Bioinspired Dual-Responsive Nanofluidic Diodes by Poly-L-lysine Modification

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ABSTRACT: A smart nanofluidic device attracts attention as it enables to control the physicochemical properties and transportation phenomena, by using stimuli-responsive materials. This work reports a bioinspired modification of a conical ion track-etched polyethylene terephthalate nanopore surface by coating a layer of poly-L-lysine (PLL), which is a commonly used coating in biotechnology to achieve a dual-responsive nanofluidic channel by pH or temperature. The rectification of ionic transportation can be reversed by assembling PLL because of the change of surface bonds from the carboxyl to amine group. The PLL-modified nanopore becomes nonconductive as an “OFF” state at pH 11.5 and at a temperature of 70 °C in solution. The ionic transport in nanopores can be switched to the “ON” (conducting) state, by either decreasing pH or temperature. The transitions between “ON” and “OFF” states present excellent reversibility, which make the PLL-modified nanopores a promising smart nanofluidic device that can be used for drug delivery or biomimetic ion/mass transport in future, besides the good biocompatibility and ease of use of PLL modification.

1. INTRODUCTION

The ion track-etched nanopores attracted broad interests because of their capability of ionic transport selectivity for the charged analytes, which can mimic the biological ion channel.1 Nanopores, nanochannels, and nanopipettes are three kinds of common solid-state pore structures.2 Solid-state nanopores are fascinating objects that enable the development of specific and efficient chemical and biological sensors, as well as the investigation of the physicochemical principles ruling the behavior of biological channels.3 The “smart” surface expanded the functionalities of the nanopores such as responsive nanofluidic diodes, sensing, and energy application,4 –8 meanwhile promoting the development of nanofluidics.9 –11 Thanks to the surface charge and conical-shape of the nanopores, ionic current has a preferential direction of conduction, comparable to a diode,12 which were used to mimic the significant physiological processes of the biological ion channels.13 –16 Thus, in a nanofluidic system, the surface properties are predominant for their physicochemical properties including rectifications of ionic current and conductance.17 In this field, particularly interesting is the development of nanofluidic channels able to precisely modify their ionic transport by selecting the values of controllable external variables.18 The “smart” surface helps to construct a functional ionic diode by using the stimuli-responsive materials,19,20 where the stimuli originated from the change of pH,4,16,21 temperature,22 light,7,23 molecules,24,25 specific ions,5,26,27 and electric field.16,28

Hereby, we report a bioinspired method to modify asymmetric polyethylene terephthalate (PET) nanopores by using poly-L-lysine (PLL), resulting in a dual-responsive nanofluidic diode for the “ON” and “OFF” states of the ionic transport. PLL has been widely used in biological and medical science because the PLL-coated surface has promotion of cellular adhesion and was used for microcapsule coatings and drug delivery because of its excellent biocompatibility.29,30 PLL can easily form positively charged films by physical adsorption and electrostatic assembling on various substrates, including glass,31 metals,32,33 polymers,34 and metallic oxides.35 Lin et al. recently demonstrated the rectification of concentration polarization in mesopores by partly coating PLL on the inner surface of nanopores, leading to high conductance ionic diodes and high performance osmotic power.36 Furthermore, it was reported that PLL can change its wettability by virtue of a dual responsive conformational change when increasing the ambient temperatures.37 We found

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that current rectification of a single nanopore can be reversed by coating of PLL, which can be controlled by the pH value of the solution because of the change of surface charge density. The amino group at the PLL surface enables to tailor the surface property of nanopores for a broad range of chemical/biomolecules. Most importantly, we found that the conduction of nanopores can be turned “OFF” by pH and temperatures (over 70 °C) with excellent repeatability because of the responsive conformational change of PLL from hydrophilic α-helix conformation to hydrophobic β-sheet conformation. Finally, we use the dual-responsive (pH and thermal) properties for future applications, like drug delivery or molecule motors, by simply coating a layer of PLL. The biocompatibility and ease-to-use help expand the advanced applications for nanofluidic devices. In this work, we developed a simple PLL coating method to construct a multifunctional nanofluidic diode, we believed that this method can be widely used in drug delivery, sensing, and smart nanofluidic transportation systems.

2. RESULTS AND DISCUSSION

Many kinds of polymers or polyelectrolytes can be functionalyzed on to the inner surface of nanopores by noncovalent bonds.PLL can be easily assembled on the inner surface of negatively charged nanopores during the strong electrostatic interaction with hydrophilic and abundant positively charged PLL. We coated a stabilized layer of PLL on the inner surface of the nanopores by immersing it in PLL aqueous solution for 4 h. The tailor chemistry and responsive wettability of the amphotheric PLL enables to construct functional nanofluidic devices. We performed X-ray photoelectron spectroscopy (XPS) analysis on a coated PET surface to ensure the success of PLL modification (Figure S3). We found that no apparent peak is observed in the N 1s region before modification with PLL, which is consistent with the fact that no N atom appears in the PET film. In contrast, after modification with PLL, an obvious peak centered at 397.3 eV is observed, implying the presence of the N atom in the PLL layer on the inner surface of nanopores.

The PET conical nanopores used in this work have a dimension of ~2 μm diameter at the base and a tip diameter of ~40 nm, characterized by SEM for the base opening using multipore membranes and conductance in 1 M KCl for the tip opening. The schematic process of PLL modification is illustrated in Figure 1a, where the blue color represents the coated PLL layer. The inner surface of nanopores before modification presents carboxy groups, which are likely to dissociate H⁺ in aqueous solution, thus forming a negatively charged surface. Whereas the modification of PLL changes the inner surface of nanopores to amine groups, which are protonated in a neutral solution, leading to a positively charged surface, and thus the inverse of current rectification (Figure 1b,c). As a result, we can adjust the current rectification in a wide range compared to a bare nanopore, by the PLL-modified nanopore. Here, we define the rectification ratio (fr) as fr = I(−V)/I(+V). To clearly represent the direction of current rectifications, logarithm r = log (fr) was introduced as a specific factor. When r > 0, the nanopore is cation-selective; however, when r < 0, the nanopore is anion-selective. We found that the r value changes from 1.72 to 0.84 with pH increases from 3 to 11.5, which we attributed to the polarity inversion of charge density at the PLL-modified nanopore inner surface. The wide range of current rectifications in PLL-modified nanopores, tuned by the pH values of aqueous solution, again demonstrated the predominant contribution of the surface charge in the nanofluidic system. The pH value of KCl aqueous solution was carefully adjusted by diluted HCl or KOH solutions. According to the reported pK∞ value of PLL (10.5), the PLL-modified nanopore has a positively charged surface at a pH lower than 10.5 while the neutral surface is at a pH higher than 10.5.

The chemical equilibrium states of the PLL-coated nanopores under different pH values are shown in Figure 2a. The I−V curves under different pH are shown in Figure 2c. In the range of 3 < pH < 10.5, the r value remains negative with an increased absolute value of the pH value, which was attributed to the gradual protonation of amine groups on the PLL layer. It is interesting to be noted that we observed a reversed rectification when pH > 10.5 (r > 0), which we suspect was due to the negative charges arising from the underlying carboxylate groups on the nanopore walls. This result is consistent with the phenomenon reported in a previous work. Our results indicate that the current rectification of a PLL-modified nanopore is responsive to the pH value of solution, with the most negative r value being −1.72 at pH = 3 and the most positive r value being 0.84 at pH = 11.5.

Besides the biocompatibility, another benefit of using the PLL-modified surface is that it is easy to be tailored. Here, we took the SA as an example which can transform amine groups into carboxyl groups in the nanopore. The scheme in Figure 2a presented the reaction process that transforms amine groups into carboxyl groups on the PLL layer. The chemical equilibrium state of the PLL layer after reacting with SA is also illustrated in Figure 2b. The reaction with SA transforms the exposed amine groups into carboxyl groups, which are negatively charged when pH > 3. The responsive behaviors of the PLL nanopores after reacting with SA was demonstrated in Figure 2b. The pH value of solution was increased from 3 to 8.6, which was attributed to the gradual deprotonation of carboxyl groups. These experiments illustrate the versatility of the PLL layer in combination with the chemistry of the amine group for tailoring the surface properties of pore walls.

![Figure 1](image-url)
It was reported that the wettability can be changed on a PLL-modified surface because of the conformational change of PLL because of the high temperature and pH.  

By using this pH and thermal responsive wettability change, we could possibly achieve “ON” and “OFF” states of the ionic conduction by virtue of a dual responsive conformational change.

Figure 3a illustrated that the schematic conformation of PLL was α-helix at room temperature (20 °C) when pH = 11.5 (higher than pK_a of PLL 10.5), which remained as a hydrophilic wetting state of the nanopore inner surface. However, the conformation changed to the β-sheet at a temperature of 70 °C possibly inducing a hydrophobic surface that repels water from the nanopores.

To investigate the thermal responsive effects on the ionic conductivity of the PLL-modified nanopores, we gradually increased the temperature of the electrolyte solution (pH = 11.5) by placing the whole setup on a heating stage, with the solution temperature characterized by a thermometer. The I−V curves of PLL-modified nanopores in 0.1 M KCl solutions of different pH after reacting with SA.

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temperature. However, when the ambient solution temperature increases to 70 °C, we found the PLL-coated nanopores become nearly nonconductive, probably caused by the transition of wettability from hydrophilic to hydrophobic corresponding to the conformation transforming of the PLL layer from α-helix to β-sheet. When pH = 11.5, temperature = 20 °C, the conformation of the PLL layer was α-helix, which was hydrophilic, the KCl solution can transport through the nanopore. This is the “ON” state. While the temperature increased to 70 °C, the conformation of the PLL layer changed to the β-sheet, which was hydrophobic, the KCl solution cannot transport through the nanopore. This is the “OFF” state. Therefore, we could tune the artificial ionic channel to be the “ON” state by maintaining the hydrophilic α-helix conformation at low temperatures, and turned it “OFF” by increasing the temperature to hydrophobic β-sheet conformation. As a control group, we performed the I−V characterizations of a bare nanopore in the same conditions (0.1 M KCl solution at pH = 11.5) under different temperatures (Figure 3c). We found that the conductance continuously increased even at 70 °C, with the increased value of nanopore conductance well matched to the increase of bulk conductivity at high-temperature solutions. The results from the control group experiment demonstrate the “OFF” state of the artificial ionic channel directly relevant to the coated PLL layer.

A few phenomena were interesting to be noted. First, to clearly discuss the effects of the temperatures, we again derived the rectification ratio (Figure 3e) and conductance (Figure 3f) by the slope of I−V curves at positive and negative biased voltages. Figure 3f indicated the conductance increased with the temperature from 20 to 50 °C at the “ON” state, which can be attributed to the increase of bulk conductivity of KCl solutions regarding the increase of temperatures. The increased \( f_{\text{rec}} \) in PLL-modified nanopores seemed to be higher than in the bare nanopore, which can be seen in Figure 3e with rectification ratio \( f_{\text{rec}} \) as a function of temperature. Our results showed that the \( f_{\text{rec}} \) increased from 6.99 to 10.4 corresponding to the temperature of solution increasing before it transformed to the “OFF” state (below 70 °C). However, the \( f_{\text{rec}} \) remained almost the same for the bare nanopore at all temperatures because of the equal enhancements of conductance at positive and negative biased potentials. We suspect that the surface charge increased with the gradual conformational change of the PLL surface. Hence, we suspect that the clear enhancement of rectification ratio was due to the increase of surface charge at the chemical equilibrium state of modified PLL caused by conformational change with increase of temperature.

The transition of PLL conformation was reported at the solution of high pH values and high temperatures, which make it possible to be switched between “ON” and “OFF” states of an artificial ionic channel by changing both pH value and temperature. To prove this concept, we performed the experiment with control experiments of PLL-modified nanopores in pH = 7 solution at a temperature of 70 °C. We characterized the I−V curves of PLL-modified nanopores in 0.1 M KCl solution (pH = 7) at different temperatures (Figure 3d), and found that the conductance continuously increased with the temperature from 20 to 70 °C. This result indicated that the switch of the “ON/OFF” state could be regulated by both pH and temperature.

In addition, hereby we performed heating and cooling cycles on the solution (Figure 3g) and measured the I−V curves of PLL-modified nanopores, maintaining the pH value at 11.5. Our results in Figure 3g demonstrated the ionic conduction can be turned to be conductive (ON state) and nonconductive (OFF state) with an excellent reversibility switched by changing the temperature between 20 and 70 °C in 0.1 M KCl (pH = 11.5). Besides, the rectified currents existed at 20 °C when it was a “ON” state and disappeared at 70 °C when it was an “OFF” state. Our results indicate that the versatile functions of PLL-modified nanopores, which act as a dual responsive ion gate that can be reversibly switched by the temperature and pH of the solution. Finally, we believe that the PLL-modified nanopores can be useful in smart control of ion/mass flow in drug delivery, chemical/biosensing, and transportation-related phenomena.

3. CONCLUSIONS

We report a bioinspired strategy to achieve a dual-responsive functional nanofluidic device by employing a layer of PLL on a conical single nanopore surface. With the PLL successfully self-assembled on the inner surface of nanopores because of physical/electrostatic adsorptions, we demonstrate that the PLL-modified nanopores has an inverted rectification compared to the initial state because of the change of the surface charge polarity, resulting in a pH-dependent nanofluidic diode. Furthermore, the PLL layer can be further modified by SA that transform the amino groups into carboxyl groups at the inner surface, leading to the inverse of surface charge. The modification of the PLL layer enables further condensation reaction based on amino and carboxy groups. Furthermore, we demonstrated a dual-responsive ionic transport by using the transition of wettability at PLL-modified surface by either pH or temperature. Our results showed that the ionic conduction of a single nanopore can be switched “OFF” (nonconductive) at pH 11.5 and in 70 °C solutions; however, it can be switched “ON” (conductible) by either lowering the temperature or pH value. We attribute this transition of nanopore conduction to the conformation change between hydrophilic (α-helix) and hydrophobic (β-sheet) of the PLL layer, in which the former state repels water and ions transported through the nanopores. We found that this transition between “ON” and “OFF” states is reversible and can be well repeated for many cycles. Finally, we achieved a pH and temperature responsive ionic conduction in a single conical nanopore, enabling well-controlled ion/mass transportation within the nanopores. We believed that the PLL coatings can be widely useful in the drug delivery, sensing, and smart nanofluidic transportation systems.

4. EXPERIMENTAL SECTION

4.1. Fabrication and Modification of a Single Nanopore. The 13 μm PET foil was irradiated by a single swift heavy ion \(^{86}\text{Kr}\) of 2.1 GeV at the Lanzhou Interdisciplinary Heavy Ion Microbeam to form a single latent track and then the foil was irradiated under ultraviolet (365 nm, 4.2 mW/cm\(^2\)) for 1 h on both sides. The foil was subjected to a standard asymmetric wet chemical etching process which was described previously.\(^{36,47}\) The foil with a single conical nanopore was immersed in a 25 μg/mL PLL solution (M\(_\text{w} = 150,000−300,000\), Sigma-Aldrich) for 4 h, then was rinsed with ultrapure water, and dried in an oven at 45 °C for 4 h before measurements.\(^{35}\)

4.2. Current Measurements. The single conical nanopore PET foil was mounted on a custom-designed system for

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**Note:** The text contains references to figures and equations that are not visible in the text. The full context and experimental details are provided in the original document.
current measurements, where the foil isolated the two chambers containing 0.1 M KCl solution. Current–voltage characteristics were recorded with a picomammeter (Keithley 6487) with a pair of Ag/AgCl electrodes. The voltage was changed between −1 and +1 V with 0.1 V steps. During the temperature responsive experiments, the whole measurement system was placed in a water bath to keep the ambient temperatures constant, and to obtain I–V curves at the meantime.

**ASSOCIATED CONTENT**

- **Supporting Information**
  The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03850.
  - XPS data, SEM image, and some electrochemical results of the nanopores (PDF).

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