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DNA-Modified Polymer Pores Allow pH- and Voltage-Gated Control of Channel Flux

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Supporting Information

ABSTRACT: Biological channels embedded in cell membranes regulate ionic transport by responding to external stimuli such as pH, voltage, and molecular binding. Mimicking the gating properties of these biological structures would be instrumental in the preparation of smart membranes used in biosensing, drug delivery, and ionic circuit construction. Here we present a new concept for building synthetic nanopores that can simultaneously respond to pH and transmembrane potential changes. DNA oligomers containing protonatable A and C bases are attached at the narrow opening of an asymmetric nanopore. Lowering the pH to 5.5 causes the positively charged DNA molecules to bind to other strands with negative backbones, thereby creating an electrostatic mesh that closes the pore to unprecedentedly high resistances of several tens of gigaohms. At neutral pH values, voltage switching causes the isolated DNA strands to undergo nanomechanical movement, as seen by a reversible current modulation. We provide evidence that the pH-dependent reversible closing mechanism is robust and applicable for nanopores with opening diameters of up to 14 nm. The concept of creating an electrostatic mesh may also be applied to different organic polymers.

Stimuli-responsive channels and pores are widespread in nature and serve as an inspiration to build biomimetic smart nanoporous membranes for applications in drug delivery or sensing.[1−9] Voltage or pH responsiveness is achieved in biological channels via gates that sense the physicochemical triggers and undergo a nanomechanical change to alter the effective pore diameter.[10−12] Replicating the stimulus-responsive behavior with more robust chemical building blocks is, however, challenging because of the structural complexity of the biological protein channels. Structurally simpler artificial systems have been built by installing a nanomechanical gate composed of negatively charged DNA strands at the pore entrance to affect their voltage-dependent movement into or out of the channel.[13] Furthermore, a pH-responsive gate was obtained by filling a pore with organic polymers that swell or shrink in response to pH.[14−18] A related concept was implemented with DNA molecules that undergo a pH-triggered conformation from folded and compact to disordered and extended.[19] In these studies, the change in pore flux was up to a factor of 10, but to date it has not been possible to achieve complete yet reversible pore closure as indicated by a high resistance of several gigaohms. This would be particularly relevant for nanopores with diameters below 10 nm, which are very attractive in a variety of applications, including drug release.[1,7−9,20] Furthermore, to date it has not been possible to create pores that respond to both voltage and pH. Here we describe the generation of DNA-modified polymer pores that introduce a new concept of nanomechanical gating. The pores offer pH- and voltage-gated control, which helps to completely switch off the channel flux as well as to tune the preferred direction of ion current flow. The concept of the nanomechanical gate is generic and may be realized with many polymers other than DNA.

Our artificial, gated channels are composed of conical polymer pores modified with DNA strands that serve as voltage and pH sensors. The pores are cone-shaped and feature a narrow opening. Figure 1 shows how negatively charged DNA acts as a voltage sensor by moving into and out of the narrow pore opening in response to the externally applied potential. The presence of the DNA at the narrow opening (Figure 1a,b) exerts two opposing effects on the ion current: steric by partial pore blocking, as shown in Figure 1a, and electrostatic (enhancing) as a result of additional cations brought to the pore to fulfill electroneutrality (not shown).[1,21,22] In addition to voltage gating, a DNA-modified nanopore can be rendered pH-responsive (Figure 1c,d). In order to induce modulation by pH, the oligonucleotide sequence has to be designed to include protonatable nucleobases so that a mildly acidic pH will produce DNA strands with positively charged bases. We hypothesize that the presence of such local positive charges will promote electrostatic attraction to the negatively charged backbone of neighboring DNA strands and the subsequent formation of an electrostatic mesh that causes pore closure (Figure 1c,d).

The experiments to implement and test our new concept of pH and voltage gating were performed with single tapered, cone-shaped nanopores prepared in a polyethylene terephthalate film by the track-etching technique.[23,24] Their limiting narrow diameter was determined in 1 M KCl by an electrochemical method as described previously.[23] The validity of the sizing method was confirmed by independent approaches involving (i) conductance measurements in the presence of poly(ethylene glycol)[25] as used in electrophysiology,[26] (ii) measurements of surface conductance,[27] and (iii) a template method involving

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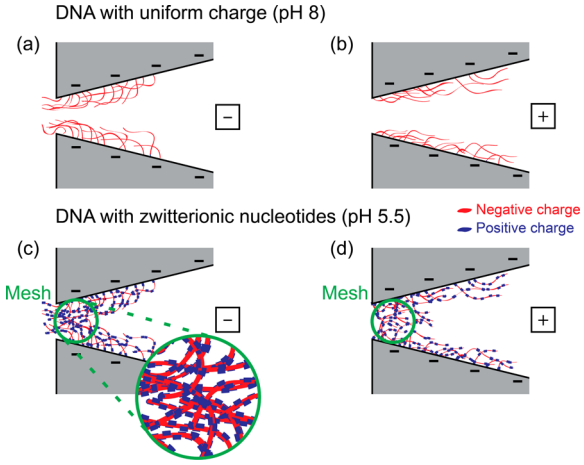

Figure 1. Schematic of voltage and pH gating in nanopores modified with DNA oligomers. (a) Voltage-induced deflection of negatively charged DNA strands from the negative potential (indicated by the boxed minus sign) toward the smaller pore opening partly obstructs the channel. (b) A potential of opposite polarity reverses the blockade by triggering DNA movement toward the larger pore opening. (c) Lowering the pH to 5.5 leads to the formation of an electrostatic mesh, closing the pore. (d) Voltage-induced deflection of oligomers toward the wide opening leads to partial pore opening. The numbers of positive charges in (c) and (d) do not reflect the actual charge density.

deposition of metal within the pore, dissolution of the polymer matrix, and imaging of the resulting metal cone.[13] Single-stranded DNA molecules with a length of 30 nucleotides were attached at the tip of a pore.[21,27,28] The coupling was achieved by activation of the carboxyl groups on the pore walls to form esters and subsequent reaction with the amino group present at the 5′ end of the DNA oligomer to form an amide linkage. The oligomer carried a C_{12} linker between the amino group and the oligomer to enhance the flexible movement of DNA in the pore. In order to create an oligomer with local positive charges, we tailored the DNA sequence of the oligomers considering pK_a values of the bases. Adenosine (A) in DNA has a pK_a of around 5.3,[29] similar to that of cytidine (C),[30] while the value for guanosine (G) is below 3 and thymidine (T) is usually not protonated.[31] Lowering the pH to 5.5 will hence cause AC but not GT DNA strands to be protonated to create local positive charges. Consequently, our model predicts that nanopores modified with AC strands will close for ionic transport at pH 5.5 as a result of the formation of an electrostatic mesh (Figure 1c). By contrast, pores containing only G and T are not expected to show pH gating; solely the negatively charged backbone will cause voltage gating. To verify these predictions, we produced pores with either AC or GT DNA as well as a combined mixture of all four bases.

Figure 2 shows example current−voltage (I−V) curves of a nanopore with a narrow opening of 8 nm modified with a DNA strand with the sequence 5-CCA CAA CCA CCA ACA CAC ACC ACC ACC-3. At pH 8, the DNA-modified nanopore was characterized by an increased conductance compared with the values before DNA attachment (Figure 2). The data suggest that the additional cations brought by DNA overcame the steric effect of the strands in this pore. The higher current magnitudes at negative voltages than at positive voltages, also known as rectification, are characteristic of our conical and other asymmetric pores.[23−25,27,32−35] Lowering the pH to 5.5 dramatically decreased the pore conductance by ∼60-fold to

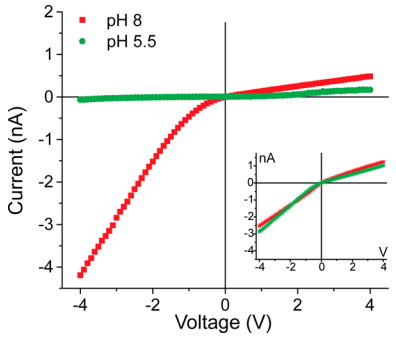

Figure 2. Current−voltage curves of a nanopore modified with AC-rich oligomers recorded in 100 mM KCl at pH 8 (red) and pH 5.5 (green). The small opening of this pore after DNA attachment was 8 nm. The inset shows the curves for the same pore before DNA coupling. The data are averages of three scans. Lowering the KCl concentration to 10 mM reduced the currents to values below 20 pA for both voltage polarities (Figure S1a,b).

below 100 pA at −4 V (Figure 2), suggesting physical pore closure in line with our model for pH gating (Figure 1).

The formation of the proposed pH-induced electrostatic biopolymer mesh was tested by varying the pore diameter. Figure 3 summarizes the pH-dependent ionic current values for six

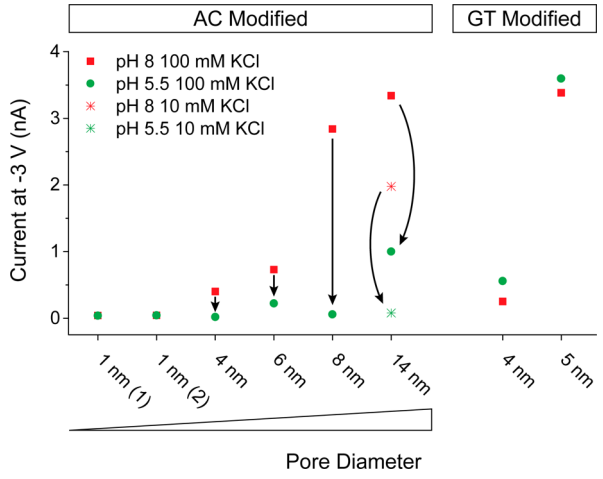

Figure 3. Ionic current recorded at −3 V for independently prepared nanopores after attachment of (left) AC-rich DNA and (right) a control oligomer containing GT. Points in red and green present data recorded at pH 8 and pH 5.5, respectively, for voltage scans from −4 to +4 V. The opening diameters for all of the pores were calculated after DNA attachment on the basis of current−voltage curves recorded at 1 M KCl and pH 8. Under these conditions, DNA is known to assume a compact random coil configuration.[36] A semilogarithmic version of the plot is shown in Figure S3a. Example I−V curves of the nanopores are shown in Figures S6 and S7.

nanopores with effective diameters ranging from 1 to 14 nm carrying AC DNA strands. In line with expectations, the initial conductance at pH 8 scaled with the diameter of the nanopore; nanopores with opening diameters below 4 nm were already occluded with DNA at pH 8. The striking feature is the great reduction in the ion current at pH 5.5, which was roughly independent of the initial conductance at pH 8. This observation points to the existence of a general and robust mechanism responsible for the pore closure. The pore closure for the 14 nm pore was less dramatic because the pore-wall-tethered DNA strands might not have been effectively long enough to bridge the

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pore gap under the experimental conditions of 100 mM KCl. However, the pore completely closed at 10 mM KCl, where DNA molecules are known to be more extended.[21,36] The formation of an electrostatic mesh is also supported by confirming the reversibility of the pH-induced current blockades. When the pH was repeatedly alternated between 8 and 5.5, switching between open and closed states was observed for pores modified with AC-rich oligomers (Figure S2 in the Supporting Information). The concept of pH-dependent switching was also observed for double-stranded DNA (Figure S1c,d).

As further evidence of the mesh formation, Figure 3 summarizes experiments with a control single-stranded DNA sequence consisting of G and T. As expected for these poorly protonatable nucleobases, the corresponding pores did not close in response to a drop in pH. The minor change in the current values is due to electrostatic factors. According to our earlier studies of conical nanopores, modulation of the pore surface charge density has a nontrivial, nonmonotonic influence on the ion current and rectification, which are also dependent on the details of pore geometry.[37] Thus, various pores before DNA attachment and after GT-rich modification showed a small positive or negative change of the current when the pH was switched from 8 to 5.5. The lack of a major pH responsiveness was certainly due to the presence of only nonionizable GT bases because DNA with all bases adopted the reversible pH-dependent blocking (Figures S3 and S4) as seen also for AC DNA. A final line of evidence for the formation of a DNA mesh at the pore tip is provided by the lack of DNA-induced gating for a pore where DNA was attached at a distance of a few hundreds of nanometers from the narrow opening (Figure S5). Since this pore has a DNA-free tip, the nucleic acid strands could not effectively span the wider distant pore lumen.

Detailed analysis of $I-V$ curves of DNA-modified nanopores revealed molecular details for the conductance change and provided further evidence for the synergistic effects of voltage and pH on ionic transport. Figure 4 shows $I-V$ curves for a pore with an effective opening diameter of 1 nm. Since this small pore became occluded with DNA even at pH 8 (Figure 4a), lowering the pH to form an electrostatic mesh did not significantly affect the recorded ionic current (Figure 4b). However, the current trace confirms the free versus restricted movement of DNA at the two pH values, as shown by the dependence of the $I-V$ curves on the direction of voltage scanning. At pH 8, the forward bias from −4 to +4 V led to lower current values compared to the reverse bias, which also caused a large unsteady deviation (Figure 4a). As discussed previously,[21] this hysteresis originates from the flexibility of the DNA strands, whose conformation is controlled by the applied voltage and the ionic concentration in the pore. Lowering the pH to 5.5 abolished the hysteresis (Figure 4b), in line with the proposed formation of a mesh, which greatly reduces the conformational flexibility of the DNA strands.

Figure 4 also establishes a conductance change that is highly unusual for conical nanopores. Usually, chemically unmodified asymmetric pores with negatively charged pore walls are characterized by current rectification (i.e., higher currents at negative voltages), which is caused by electrostatic factors.[23,24,32−35] However, the DNA-modified pores display the opposite behavior, with currents for positive voltages higher than for negative voltages (Figure 4a, forward scan (−4 V to 4 V); Figures S4 and S6). Although flipping of the $I-V$ curve asymmetry has been achieved previously by coupling of positively charged molecules to the pore wall,[27,34,38,39] the inversion of $I-V$ rectification by adding extra negative charges

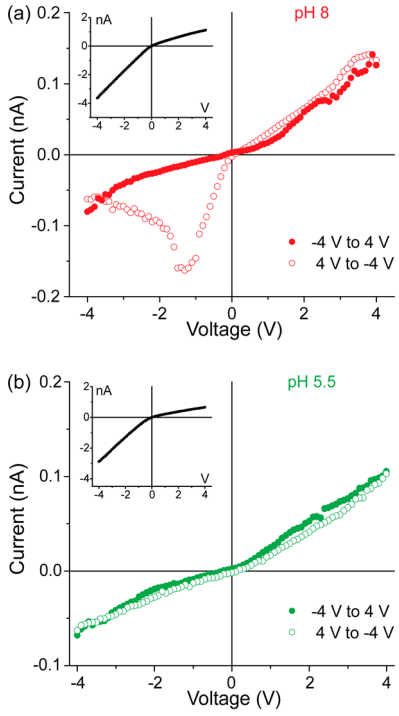

Figure 4. Current−voltage curves recorded for a 1 nm diameter pore at (a) pH 8 and (b) pH 5.5. The opening diameter of this pore before DNA attachment was 3 nm; transport properties of the unmodified structure under the same conditions are shown as insets.

(in the form of DNA strands) is new. The phenomenon becomes even more pronounced at pH 5.5 (Figure 4b) and occurs for all AC-modified nanopores shown in Figure 3, except the one with 14 nm diameter (Figure S6). The inversion of the $I-V$ rectification and similar ion current values at both pH values can be explained by assuming that the DNA mesh exerts a very large steric effect and thereby dominates the surface-charge-driven electrostatic factors that usually cause regular rectification. Electrostatics and voltage may, however, play a role at pH-gated pores, albeit minor, as shown by the larger currents at positive potentials at pH 5.5 (Figure 4b and Figures S4 and S6). The higher currents likely reflect the fact that at positive voltages the overall negatively charged zwitterionic strands are deflected toward the wide pore opening, resulting in a loosened mesh (Figure 1d), which enables a higher ionic current flow. In the interpretation of the data, electroosmosis was not considered since its effect on ion current rectification in nanopores was shown to be very small.[40]

In summary, we have presented a new concept capable of achieving voltage and pH gating of pores with tethered single-stranded DNA strands. Reversible voltage-controlled nanoscale movement of the voltage sensors is complemented by reversible protonation of the pH sensors to form a mesh stabilized by electrostatic interactions. We expect that our new principle of regulating the effective pore diameter can be implemented with other ionizable organic polymers, thereby opening up applications in, for example, drug delivery. Our pores are also the first synthetic nanoporous system whose direction of rectification is regulated by attached electrochemical gates.

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■ ASSOCIATED CONTENT

Supporting Information

Procedure for DNA attachment, ion current measurements, and $I-V$ curves for nanopores modified with DNA oligomers of three difference sequences. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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