Published in final edited form as:

ACS Nano. 2011 October 25; 5(10): 8404–8411. doi:10.1021/nn203205s.

Single Nanopore Investigations with Ion Conductance Microscopy

Chiao-Chen Chen, Yi Zhou, and Lane A. Baker*

Department of Chemistry, Indiana University, 800 E. Kirkwood Avenue, Bloomington, Indiana 47405

Abstract

A three-electrode scanning ion conductance microscope (SICM) was used to investigate the local current-voltage properties of a single nanopore. In this experimental configuration, the response measured is a function of changes in the resistances involved in the pathways of ion migration. Single nanopore membranes utilized in this study were prepared with an epoxy painting procedure to isolate a single nanopore from a track-etch multi-pore membrane. Current-voltage responses measured with the SICM probe in the vicinity of a single nanopore were investigated in detail and agreed well with equivalent circuit models proposed in this study. With this modified SICM, the current-voltage responses characterized for the case of a single cylindrical pore and a single conical pore exhibit distinct conductance properties that originate from the geometry of nanopores.

Keywords

Microscopy; nanopore; conductance; membrane and ion transport

As the size of a pore shrinks, new physical properties can be observed due to the high surface area-volume relationship.¹ Particularly at the nanoscale, the electrical double layer,^{2–5} surface conductivities,^{6–8} and geometric asymmetry^{9–10} can result in new properties not observed at larger scales. Studies of transport at these small scales – particularly at the level of single nanopores – are fertile ground to develop deeper knowledge of the fundamentals of physical and biophysical processes.^{11–13} Further, applications of nanopores in the venues of chemical separations^{14–17} and sensor development^{18–22} promise advances in fields as diverse as genetic sequencing^{23–27} and fuel cells.^{28–32} Thus, new tools to study nanopores and nanoscale transport may find significant impact in the realm of both fundamental and applied sciences. Previous applications of scanning probe microscopies to the study of nanopores have demonstrated utility in determination of conductivity pathways and measurement of local concentrations or conductivities in the vicinity of the nanopore.^{33–39} Herein, we describe a modified three electrode ion conductance microscope and demonstrate measurement of the properties of a single pore. With this configuration, the current-voltage properties of individual pores can be interrogated while scanning or at fixed positions. Measurements of the conductance properties of a single cylindrical pore and a single conical pore are demonstrated.

* Author to whom correspondence should be addressed: lanbaker@indiana.edu; Phone: (812) 856-1873; Fax: (812) 856-8300.

Supporting Information Available:

Comparison of the characteristics between three-electrode SICM reported here and the conventional two-electrode SICM system is discussed in supporting information. In addition, characterizations of nanopipettes and single nanopore membranes utilized here as well as the finite element method (FEM) modeling for three-electrode SICM are described. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Scanning ion conductance microscopy (SICM) scans the surface of a sample with a nanometer scale pipette (nanopipette), often to collect topographic information in a non-invasive fashion.^{40–45} In SICM, the position of the pipette is controlled by carefully monitoring a small gap between the sample and pipette. The resistance of this gap (termed an access resistance) is a strong function of tip-sample separation, and provides a method for feedback.^{46–50} In addition to feedback control, under certain imaging conditions or scanning modes, ion currents from the pipette can also be monitored directly to characterize properties of the sample.^{41, 51–54}

Here, membranes with single pores (cylindrical and conical in shape) have been prepared *via* the track-etch method.⁵⁵ A modified scanning ion conductance microscope with an additional electrode that serves to drive ions through this single pore is employed to examine nanopore conductivity. We find that for the case of a single pore, this three electrode system results in a predictable interaction between the electrode of the SICM and the working electrode of the nanopore. Advantages of this three-electrode configuration over a more conventional two-electrode SICM include independent control of the cross-membrane potential and enhanced contrast in measurement of nanopore conductance. (A detailed description of the experimental advantages is provided in the supporting information.) Further, through careful control of pipette position (relative to the membrane pore) and control of the working electrode potential, the current-voltage response for the pore in the membrane can be measured with the pipette. Nanopipettes were also capable of “dipping into” membrane pores, to operate within the pore itself.⁵⁶

A model electrical circuit is proposed that predicts the observed response. Additional finite element simulations further confirm the proposed mechanism. The response for a cylindrical pore is described in detail and measurement of a conical nanopore with rectified current flow is demonstrated. These preliminary results demonstrate that this three-electrode SICM provides a platform to characterize individual nanopores independent of other structures involved in the same system. Ultimately, this tool will provide a method to monitor nanopores in more complex biological and sensing environments.

Results/Discussion

Current-voltage responses measured with modified SICM

Single nanopores were isolated, optical and electron micrographs of one such pore are shown in Figure 1. Effects of cross-membrane potentials on the topography and current images measured by SICM for single nanopore membranes were recorded with distance-modulated (AC) feedback. The modified three-electrode SICM employed (Figure 2) allows for independent potential control of the working electrode (WE) and pipette electrode (PE), as referenced to a common reference electrode (RE). For all experiments reported in this study, the pipette electrode (PE) was held at +0.1 V and the reference electrode (RE) served as ground. Topography and the corresponding current images obtained with three different working electrode potentials (+0.3 V, 0 V and −0.3 V) are shown in Figure 3. Comparison of these three current images reveals that when the nanopipette scans over a pore, a negative working electrode potential (−0.3 V applied here) increases the potential drop across the pipette electrode (PE) and thus produces an increase in the pipette current. This results in an enhancement (greater current) of the ion current image which is obtained by plotting the changes in pipette current (ΔI) in x-y coordinates, as shown in Figure 3c. In contrast, when the working electrode (WE) was held at +0.3 V, the magnitude of the current image (ΔI) reduced to negative values (Figure 3d), as a result of reduction in the pipette currents due to the decrease of the voltage drop across the pipette electrode (PE).

To better investigate the effect of the migration currents under various working electrode potentials, instead of scanning the pipette, which can complicate the interpretation of recorded signals,^{51–52} ion currents were measured with the SICM probe at defined locations with the pipette in a fixed position. Figure 4a represents the relationship between the working electrode potential and currents measured at a vertical probe-sample distance (D_{ps}) of 170 nm. Here the probe is laterally positioned right over (Figure 4a, ■) and far away (Figure 4a, ●) from the center of a single nanopore as a function of the potential applied to the working electrode (WE). When the SICM probe was positioned over the pore center, the slope of the current-voltage response measured was about 10 times larger than that obtained when the probe was located at a lateral displacement of 8.5 μm away from the pore center.

This phenomenon is proposed to originate from changes in the resistance involved in the pathway of migration currents and can be explained well with the circuit models illustrated in Figure 2. To clarify, the access resistances R_{acc} and R'_{acc} in these two circuits have different arrangements and are related to different migration pathways. In Figure 2c, R_{acc} is the access resistance associated with the migration pathway between the working electrode (WE) and the pipette electrode (PE) due to the gap between the SICM probe and the membrane surface. However, when the nanopipette is positioned right over the pore center, the access resistance, R_{acc} , from the probe-sample distance is gone. R'_{acc} , shown in Figure 2d is generated from the migration pathway between the working electrode (WE) and the reference electrode (RE) and considers the border of the nanopore and the outer edge of the glass wall of the nanopipette.

For the probe-sample distance of 170 nm utilized to obtain Figure 4a, the magnitudes of R_{acc} and R'_{acc} in the circuit models were estimated to be 1 $\text{M}\Omega$ and 0.75 $\text{M}\Omega$, respectively. Numerical values for R_{acc} and R'_{acc} at D_{ps} equal to 170 nm were assessed from approach curves. The approach curves were obtained by recording changes in the absolute pipette current as the probe was approached from bulk electrolyte to the membrane and to the pore center, respectively. For determination of access resistances, 0 V was applied to the working electrode (WE). Typical values of $R_{pipette}$ and R_{pore} are 100 $\text{M}\Omega$ and 86 $\text{M}\Omega$, respectively, as mentioned in the experimental section. Compared to these resistances, additional resistances associated with the electrolyte solution (0.1 M KCl) and the charge transfer rate of Ag/AgCl electrodes, R_{sol} and $R_{Ag/AgCl}$, are typically negligible. Figure 4b represents the current-voltage responses simulated with the proposed circuit models, which fit well with the experimental data shown in Figure 4a. To further confirm observations of these position-dependent current-voltage responses detected and to estimate the capability of proposed equivalent circuit models, finite element method (FEM) simulation, was utilized to provide additional information (supporting information). Simulated results from finite element models (Figure S4) fit well with experimental measurements and predictions from the equivalent circuit models.

Spatial distribution of current-voltage responses

The current-voltage response through a single nanopore measured with an SICM probe in the vicinity of the pore opening displays a vertical distribution along the direction perpendicular to the membrane surface as shown in Figure 5a. Here, absolute pipette current was recorded as a function of the potential applied to the working electrode (WE) at five selected probe-sample distances (D_{ps}). Negative values of the D_{ps} mean that the probe (outer diameter of ~ 250 nm, cone angle of $\sim 9.5^\circ$) was moved into the nanopore (pore diameter ~ 500 nm). From these current-voltage responses, the effect of the applied potential on the measured pipette current became more apparent, especially when the probe was moved deeper into the pore center. When the probe moves into the pore, significant changes in the value of the access resistance, R'_{acc} , are observed. This is likely a consequence of enhanced interactions between the charged walls of the pipette and the pore, similar to so-called

“squeezing effects” observed in nanopore studies.^{40, 57} A more direct evidence for the increase of R'_{acc} can be realized from the decrease in the absolute pipette current with the reduction of the probe-sample distance (D_{ps}) when the working electrode was maintained at 0 V (marked with black arrow in Figure 5a).

This vertical distribution of the current-voltage responses can be reproduced with the circuit model shown in Figure 2d which describes the active components (resistors and capacitors) involved in the system when the nanopipette is positioned over the pore center. Numerical values of R'_{acc} utilized in the circuit model that correspond to various probe-sample distances (D_{ps}) were estimated based on approach curves as described previously. Simulated data from the circuit model is illustrated in Figure 5b where the current-voltage response of greatest slope was obtained with the largest R'_{acc} and related to that recorded with the most negative D_{ps} shown in Figure 5a. In contrast, when the probe-sample separation is large (D_{ps} 610 nm), the pipette current remained relatively constant independent of the potential applied to the working electrode (WE) due to the small access resistance (R'_{acc} 0.4 M Ω).

In addition to vertical distribution, current-voltage responses measured with an SICM probe at a constant probe-sample distance also demonstrate dependence on lateral displacement from the pore center. Selected current-voltage (I-V) responses recorded from 0 μm to 8.55 μm away from the pore center at D_{ps} = 170 nm demonstrate a lateral distribution shown in Figure 6a. With consideration of the geometry of the SICM probe (ID $\sim$ 60 nm, OD $\sim$ 250 nm) and the single nanopore ($\sim$ 500 nm in diameter), the probe opening and the pore opening are completely overlapped (*i.e.* R_{acc} which results from the small probe-sample gap does not exist) at lateral displacements from 0 μm to 0.18 μm and thus the behavior of I-V responses recorded are dominated by the access resistance, R'_{acc} , in the circuit model shown in Figure 2d (indicated as group 1 in Figure 6a). Responses measured at lateral displacements larger than 0.33 μm (classified as group 3 in Figure 6a) show similar and more gradual slopes. The behavior of these I-V responses can be explained with the circuit model illustrated in Figure 2c. Since the SICM probe was far from the single nanopore, the access resistance, R_{acc} , depends on the nanoscale probe-sample gap only. For the current-voltage response recorded at the lateral displacement of 0.33 μm (group 2 in Figure 6a), the tip opening of the SICM probe was moved away from the pore opening while the nanopipette still partially overlapped the single nanopore. Consequently, both R_{acc} and R'_{acc} affected the magnitude of pipette currents measured and thus resulted in an I-V relationship located between the two extreme conditions in which the current-voltage responses were governed by only R_{acc} or R'_{acc} , respectively.

From the I-V responses recorded when the SICM probe was moved laterally away from the pore center, line profiles of the current magnitude measured at various applied potentials can be reconstructed. Figure 6b illustrates the reconstructed line profiles of the pipette current measured at five representative applied potentials. Potential applied to the working electrode (WE) results in greatest changes in current magnitude when the nanopipette was located at the center of the single pore. In contrast, the magnitude of the currents measured was fairly constant under a given applied potential when the SICM probe was away from the pore center.

Characterization of pore geometry

In addition to the case of a single cylindrical pore examined in the experiments discussed above, the current-voltage response through a single conical pore was characterized with this three-electrode SICM as well. The single conical pore membrane was prepared by the epoxy painting method described in the experimental section and a conical pore with a base diameter of 690 ± 25 nm ($n = 3$) and a tip diameter of 44 ± 8 nm ($n = 3$) was isolated for this

experiment. The single conical pore membrane was mounted tip-side up on the perfusion cell in which the SICM probe was allowed to access the tip side of the conical pore.

Figure 7a shows pipette currents, measured over the center of the tip opening of the conical pore (Figure 7a, ■) and at a lateral displacement of 5.9 μm away from the pore center (Figure 7a, ●), as a function of the potential applied to the working electrode (WE) at a constant probe-sample distance (D_{ps}) of 150 nm. From this plot, differences of current-voltage responses measured with SICM that depend on pipette position can also be observed with the conical pore membrane. Similar to a cylindrical pore membrane, the magnitude of the pipette current recorded far from the pore center remained almost constant independent of the potential applied. However, the current-voltage response recorded over the conical pore center (Figure 7a, ■) showed significant difference to that obtained with a cylindrical pore membrane (Figure 4a, ■).

Conical nanopores exhibit rectified current flow, a consequence of the asymmetric pore geometry and the high surface to volume ratio of the tip. For conical nanopores with negative surface charge, a negative potential on the large (base) side of the pore results in greater currents (*i.e.* higher pore conductance/lower pore resistance) than the case of the corresponding positive potential. (supporting information, Figure S2c). Here, membranes with a single, negatively charged conical nanopore were mounted tip-side-up in the perfusion cell. The working electrode (WE) in the lower chamber faced the base side of the conical pore, with the reference electrode (RE) in the upper chamber. Therefore, a greater migration current (*i.e.* higher pore conductance/lower pore resistance) through the conical pore was observed when the working electrode (WE) was held at a negative potential compared to the case in which the corresponding positive potential was applied. Furthermore, in the case of a single cylindrical pore, when the SICM probe was located over the pore center, a linear change in pipette current induced by the potential applied to the working electrode (WE) was observed. However, for the conical pore investigated here, due to the aforementioned rectification effect, the pore resistance is not constant but changes non-linearly with the applied working electrode potential. This results in a curved (rectified) relationship between the absolute pipette currents measured and the potential applied to WE.

Therefore, in contrast to the cylindrical pore, in which changes in the absolute pipette current ($\Delta I = I_{\text{pipette, WE}} - I_{\text{pipette, WE}=0}$) provide a linear relationship to the working electrode potential, ΔI of a conical pore displays a rectified response, as shown in Figure 7b. In addition, for a cylindrical pore, negative working electrode potentials result in a positive ΔI (increase in absolute pipette current) while a negative ΔI (decrease in absolute pipette current) was detected at positive working electrode potentials. The same phenomenon was observed for the conical pore. Consequently, the direction of current rectification shown in Figure 7b is reversed to that of the bulk current-voltage response determined with conventional macroscopic measurements shown in Figure S2c. Although the rectification direction is reversed, the rectification ratio observed from the nanopipette measurement ($|\Delta I_{-0.6\text{V}}/\Delta I_{+0.6\text{V}}| = 1.83$ from Figure 7b) is in good agreement with the result for the measurement of rectification ($|I_{-0.6\text{V}}/I_{+0.6\text{V}}| = 1.84$ from Figure S2c) of the conical nanopore membrane from the bulk.

Hence, the absolute pipette current recorded as a function of potential applied to the working electrode (WE) displays a rectified current response which reflects the conical geometry of the nanopore under study. This result demonstrates that by examination of the current-voltage responses measured with an SICM probe, nanopores with distinct geometry can be identified.

Conclusion

In this study, we have developed a simple approach to prepare a single nanopore membrane that utilizes water-resistive epoxy to isolate a single nanopore from a low density track-etched polyimide membrane. This painting method allows preparation of single nanopore membranes without complicated lithography procedure. In addition, a modified scanning ion conductance microscope (SICM) with three electrodes has been established to investigate the effects of ion migration on the current magnitude measured at the vicinity of a single nanopore. Current-voltage responses detected with an SICM probe showed spatial distributions which can be explained with equivalent circuit models purposed in this study. Furthermore, by comparing the characteristics of the current-voltage responses recorded for individual nanopores, nanopores with distinct geometry can be distinguished from each other. Therefore, this three-electrode SICM provides a promising platform to study the heterogeneous multiple nanopore (nanochannel) systems found in materials and biological settings.

Experimental Section

Chemicals and Materials

Solutions were prepared with deionized water (resistivity = 18 M Ω -cm) obtained from a Milli-Q water purification system (Millipore Corp., Danvers, MA). Sodium iodide (Mallinckrodt, Philipsburg, NJ) and sodium hypochlorite (10–15% active chlorine, Sigma-Aldrich, St. Louis, MO) were utilized to prepare nanoporous membranes as described previously.⁵⁵ Potassium chloride (Mallinckrodt, Philipsburg, NJ) solutions with concentration of 0.1 M were filtered with 0.22 μ m PVDF filter membranes (Millipore Corp., Danvers, MA) and utilized as electrolyte for SICM measurements. Clear, water-resistive epoxy (Devcon, Riviera Beach, FL) was applied to isolate a single nanopore in a membrane as described (vide infra).

Membrane preparation and characterization

Nanoporous membranes utilized in this study were prepared from low density ion-tracked polyimide membranes (track density 10⁴ tracks/cm², thickness 25 μ m, it4ip, Belgium) *via* the track-etch process.⁵⁵ Immersion of ion-tracked membranes in active sodium hypochlorite solution (10–15%) at 70°C for 15 min, resulted in porous membranes containing cylindrical pores. To prepare membranes with conical pores, an ion-tracked membrane was mounted between two halves of a U-tube, in which etching solution (10–15% sodium hypochlorite solution) was filled in one half and stop solution (1 M sodium iodide) was loaded in the other half. This U-tube with an ion-tracked membrane mounted was then placed in a water-bath for 95 min at 50°C.

Porous membranes prepared were mounted on a glass slide and placed on an inverted optical microscope (Nikon TE2000, Melville, NY) where a water-resistive epoxy was applied to one side of the membrane to isolate a single nanopore (for a conical pore membrane, the epoxy painting was applied to the base side). To increase the strength of the membrane and allow the isolated single pore to contact electrolyte solution on both sides, single nanopore membranes were then masked further in clear tape.

Pore sizes of single nanopores were determined *via* current-voltage measurements and scanning electron microscopy (SEM, FEI Quanta-FEG) as described in supporting information (Figure S2). Optical and the SEM images of representative single nanopore membranes are shown in Figure 1. Figure 1c shows an electron micrograph of a cylindrical pore from an isolated single pore membrane post examination by scanning ion conductance microscopy (SICM). Minor salt deposits which remain on the inner surface of the nanopore

can be observed. For the single cylindrical pore membranes utilized in this study, the pore diameter was characterized as 462 ± 35 nm ($n = 6$). For the single conical pore membranes applied here, pore resistance was determined with current-voltage measurements and the base diameter was characterized to be 690 ± 25 nm ($n = 3$) with SEM. As described in supporting information, the pore tip diameter was determined to be 44 ± 8 nm ($n = 3$).

Instrumentation and the equivalent circuits for the experimental setup

Data was acquired with a ScanIC scanning ion conductance microscope (ionscope, Ltd., London, UK) in conjunction with an Axopatch 200B current amplifier (Molecular Devices, Union City, CA). SICM operated in both non-modulated (DC) and distance-modulated (AC) modes has been described previously.^{41–43, 58} Briefly, the SICM probe consists of a nanopipette filled with electrolyte (0.1 M KCl) and back inserted with a Ag/AgCl electrode. A Ag/AgCl reference electrode is placed in the bath electrolyte and a constant potential is applied between the pipette and the reference electrodes. The SICM probe is mounted on a three-dimensional piezo actuator integrated with a DC motor to control pipette position.

To investigate the effect of ion migration through a single nanopore on current measured by SICM, a third Ag/AgCl electrode was placed in the lower chamber of the perfusion cell (Figure 2). The perfusion cell consists of a single nanopore membrane mounted between the upper and the lower chambers of the cell which are filled with 0.1 M KCl. Pipette and reference electrodes reside in the upper chamber. Potential applied to the pipette electrode (PE) was maintained at +0.1 V, while the reference electrode (RE) was ground to a faraday cage. Potential applied to the working electrode (WE) in the bottom chamber was controlled by a function generator (Agilent 33220A, Loveland, CO) to produce a defined cross-membrane potential. This experimental setup is illustrated in Figure 2 which depicts the case of two representative positions of a nanopipette. Current-voltage responses are detected with the SICM probe at a constant probe-sample distance (D_{ps}). In Figure 2a, the nanopipette is far away from the center of the single nanopore and the circuit model utilized to describe the components involved in this configuration is shown in Figure 2c. Figure 2b illustrates the situation when a nanopipette is located over the pore center and the associated circuit model is depicted in Figure 2d.

In both circuit models, R_{pipette} is the pipette resistance determined by the geometry of the SICM probe which is typically around 100 M Ω for the glass nanopipettes utilized in this experiment. The inner and outer diameters of these nanopipettes are about 60 nm and 250 nm (Figure S1), respectively. R_{acc} and R'_{acc} are the access resistances associated with each configuration; the magnitudes of each are affected by both the probe-sample distance (D_{ps}) and the surface topography of the specimen. R_{pore} represents the resistance of the nanopore which is about 86.0 ± 12.1 M Ω ($n = 6$) for the single pore membranes prepared for this experiment. R_{sol} and $R_{\text{Ag/AgCl}}$ are the resistances associated with the electrolyte solution (0.1 M KCl) and the charge transfer rate of the Ag/AgCl electrodes whose magnitudes are typically negligible compared to the other resistances involved in the system. In addition, C_{pipette} , C_{acc} , C'_{acc} and C_{pore} represent the capacitive components due to the high surface to volume ratios of the nanopipette, the single nanopore and the nanoscale probe-sample gap, respectively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institutes of Health (NIDDK 1R21DK082990), Research Corporation for Scientific Advancement (Cottrell Scholars Award) and the American Heart Association (Scientist Development Grant). The authors greatly appreciate valuable discussions and technical support about electronic instrumentation from Mr. Andy Alexander and Mr. John Poehlman. We are also grateful to Mr. Joseph Basore for suggestions about pore isolation and Mr. Maksymilian A. Derylo for comments.

Literatures Cited

1. Schoch RB, Han J, Renaud P. Transport Phenomena in Nanofluidics. *Rev Mod Phys*. 2008; 80:839–883.
2. Dutta P, Beskok A. Analytical Solution of Combined Electroosmotic/Pressure Driven Flows in Two-Dimensional Straight Channels: 3 Finite Debye Layer Effects. *Anal Chem*. 2001; 73:1979–1986. [PubMed: 11354479]
3. Pu Q, Yun J, Temkin H, Liu S. Ion-Enrichment and Ion-Depletion Effect of Nanochannel Structures. *Nano Lett*. 2004; 4:1099–1103.
4. Pennathur S, Santiago JG. Electrokinetic Transport in Nanochannels. 1. Theory. *Anal Chem*. 2005; 77:6772–6781. [PubMed: 16255573]
5. Pennathur S, Santiago JG. Electrokinetic Transport in Nanochannels. 2. Experiments. *Anal Chem*. 2005; 77:6782–6789. [PubMed: 16255574]
6. van der Heyden FHJ, Stein D, Dekker C. Streaming Currents in a Single Nanofluidic Channel. *Phys Rev Lett*. 2005; 95:116104. [PubMed: 16197024]
7. Nguyen G, Vlassiuk I, Siwy ZS. Comparison of Bipolar and Unipolar Ionic Diodes. *Nanotechnology*. 2010:21.
8. Plecis A, Nanteuil Cm, Haghiri-Gosnet AM, Chen Y. Electropreconcentration with Charge-Selective Nanochannels. *Anal Chem*. 2008; 80:9542–9550. [PubMed: 19006338]
9. Kubeil C, Bund A. The Role of Nanopore Geometry for the Rectification of Ionic Currents. *J Phys Chem C*. 2011; 115:7866–7873.
10. Cheng LJ, Guo LJ. Nanofluidic Diodes. *Chem Soc Rev*. 2010; 39:923–938. [PubMed: 20179815]
11. Duan CH, Majumdar A. Anomalous Ion Transport in 2-nm Hydrophilic Nanochannels. *Nat Nanotechnol*. 2010; 5:848–852. [PubMed: 21113159]
12. Hou X, Guo W, Jiang L. Biomimetic Smart Nanopores and Nanochannels. *Chem Soc Rev*. 2011; 40:2385–2401. [PubMed: 21308139]
13. Hou X, Yang F, Li L, Song YL, Jiang L, Zhu DB. A Biomimetic Asymmetric Responsive Single Nanochannel. *J Am Chem Soc*. 2010; 132:11736–11742. [PubMed: 20677760]
14. Han J, Craighead HG. Separation of Long DNA Molecules In a Microfabricated Entropic Trap Array. *Science*. 2000; 288:1026–1029. [PubMed: 10807568]
15. Kuo TC, Cannon DM, Chen YN, Tulock JJ, Shannon MA, Sweedler JV, Bohn PW. Gateable Nanofluidic Interconnects for Multilayered Microfluidic Separation Systems. *Anal Chem*. 2003; 75:1861–1867. [PubMed: 12713044]
16. Garcia AL, Ista LK, Petsev DN, O'Brien MJ, Bisong P, Mammoli AA, Brueck SRJ, Lopez GP. Electrokinetic Molecular Separation in Nanoscale Fluidic Channels. *Lab Chip*. 2005; 5:1271–1276. [PubMed: 16234951]
17. Fu JP, Schoch RB, Stevens AL, Tannenbaum SR, Han JY. A Patterned Anisotropic Nanofluidic Sieving Structure for Continuous-Flow Separation of DNA and Proteins. *Nat Nanotechnol*. 2007; 2:121–128. [PubMed: 18654231]
18. Fu YQ, Tokuhisa H, Baker LA. Nanopore DNA Sensors Based on Dendrimer-Modified Nanopipettes. *Chem Commun*. 2009:4877–4879.
19. Sa NY, Fu YQ, Baker LA. Reversible Cobalt Ion Binding to Imidazole-Modified Nanopipettes. *Anal Chem*. 2010; 82:9963–9966. [PubMed: 21090777]
20. Umehara S, Karhanek M, Davis RW, Pourmand N. Label-Free Biosensing with Functionalized Nanopipette Probes. *P Natl Acad Sci USA*. 2009; 106:4611–4616.

21. Actis P, Mak A, Pourmand N. Functionalized Nanopipettes: Toward Label-Free, Single Cell Biosensors. *Bioanal Rev.* 2010; 1:177–185. [PubMed: 20730113]
22. Ali M, Yameen B, Neumann R, Ensinger W, Knoll W, Azzaroni O. Biosensing and Supramolecular Bioconjugation in Single Conical Polymer Nanochannels. Facile Incorporation of Biorecognition Elements into Nanoconfined Geometries. *J Am Chem Soc.* 2008; 130:16351–16357. [PubMed: 19006302]
23. Kawano R, Schibel AEP, Cauley C, White HS. Controlling the Translocation of Single-Stranded DNA through alpha-Hemolysin Ion Channels Using Viscosity. *Langmuir.* 2009; 25:1233–1237. [PubMed: 19138164]
24. Cockroft SL, Chu J, Amarin M, Ghadiri MR. A Single-Molecule Nanopore Device Detects DNA Polymerase Activity with Single-Nucleotide Resolution. *J Am Chem Soc.* 2008; 130:818–820. [PubMed: 18166054]
25. Ashkenasy N, Sanchez-Quesada J, Bayley H, Ghadiri MR. Recognizing a Single Base in an Individual DNA Strand: A Step toward DNA Sequencing in Nanopores. *Angew Chem Int Edit.* 2005; 44:1401–1404.
26. Ma L, Cockroft SL. Biological Nanopores for Single-Molecule Biophysics. *Chembiochem.* 2010; 11:25–34. [PubMed: 19938028]
27. Schibel AEP, An N, Jin Q, Fleming AM, Burrows CJ, White HS. Nanopore Detection of 8-Oxo-7,8-dihydro-2'-deoxyguanosine in Immobilized Single-Stranded DNA *via* Adduct Formation to the DNA Damage Site. *J Am Chem Soc.* 2010; 132:17992–17995. [PubMed: 21138270]
28. Vichi FM, Colomer MT, Anderson MA. Nanopore Ceramic Membranes as Novel Electrolytes for Proton Exchange Membranes. *Electrochem Solid St.* 1999; 2:313–316.
29. Pereira F, Vallé K, Belleville P, Morin A, Lambert S, Sanchez C. Advanced Mesostructured Hybrid Silica Nafion Membranes for High-Performance PEM Fuel Cell. *Chem Mater.* 2008; 20:1710–1718.
30. Iroji T, Kuraoka K, Yasuda K, Yazawa T, Miyazaki Y. Surface-Modified Nanopore Glass Membrane as Electrolyte for DMFCs. *Electrochem Solid St.* 2004; 7:A394–A396.
31. Liu SR, Pu QS, Gao L, Korzeniewski C, Matzke C. From Nanochannel-Induced Proton Conduction Enhancement to a Nanochannel-Based Fuel Cell. *Nano Lett.* 2005; 5:1389–1393. [PubMed: 16178244]
32. Bussian DA, O'Dea JR, Metiu H, Buratto SK. Nanoscale Current Imaging of the Conducting Channels in Proton Exchange Membrane Fuel Cells. *Nano Lett.* 2007; 7:227–232. [PubMed: 17256914]
33. Bath BD, Lee RD, White HS, Scott ER. Imaging Molecular Transport in Porous Membranes. Observation and Analysis of Electroosmotic Flow in Individual Pores Using the Scanning Electrochemical Microscope. *Anal Chem.* 1998; 70:1047–1058.
34. Bath BD, White HS, Scott ER. Electrically Facilitated Molecular Transport. Analysis of the Relative Contributions of Diffusion, Migration, and Electroosmosis to Solute Transport in an Ion-Exchange Membrane. *Anal Chem.* 2000; 72:433–442. [PubMed: 10695125]
35. Ervin EN, White HS, Baker LA. Alternating Current Impedance Imaging of Membrane Pores Using Scanning Electrochemical Microscopy. *Anal Chem.* 2005; 77:5564–5569. [PubMed: 16131066]
36. Ervin EN, White HS, Baker LA, Martin CR. Alternating Current Impedance Imaging of High-Resistance Membrane Pores Using a Scanning Electrochemical Microscope. Application of Membrane Electrical Shunts to Increase Measurement Sensitivity and Image Contrast. *Anal Chem.* 2006; 78:6535–6541. [PubMed: 16970331]
37. Gardner CE, Unwin PR, Macpherson JV. Correlation of Membrane Structure and Transport Activity Using Combined Scanning Electrochemical-Atomic Force Microscopy. *Electrochem Commun.* 2005; 7:612–618.
38. Uitto OD, White HS. Scanning Electrochemical Microscopy of Membrane Transport in the Reverse Imaging Mode. *Anal Chem.* 2001; 73:533–539. [PubMed: 11217758]
39. Uitto OD, White HS, Aoki K. Diffusive-Convective Transport into a Porous Membrane. A Comparison of Theory and Experiment Using Scanning Electrochemical Microscopy Operated in Reverse Imaging Mode. *Anal Chem.* 2002; 74:4577–4582. [PubMed: 12236372]

40. Bocker M, Muschter S, Schmitt EK, Steinem C, Schaffer TE. Imaging and Patterning of Pore-Suspending Membranes with Scanning Ion Conductance Microscopy. *Langmuir*. 2009; 25:3022–3028. [PubMed: 19437710]
41. Hansma PK, Drake B, Marti O, Gould SAC, Prater CB. The Scanning Ion-Conductance Microscope. *Science*. 1989; 243:641–643. [PubMed: 2464851]
42. Korchev YE, Bashford CL, Milovanovic M, Vodyanoy I, Lab MJ. Scanning Ion Conductance Microscopy of Living Cells. *Biophys J*. 1997; 73:653–658. [PubMed: 9251784]
43. Shevchuk AI, Gorelik J, Harding SE, Lab MJ, Klenerman D, Korchev YE. Simultaneous Measurement of Ca^{2+} and Cellular Dynamics: Combined Scanning Ion Conductance and Optical Microscopy to Study Contracting Cardiac Myocytes. *Biophys J*. 2001; 81:1759–1764. [PubMed: 11509385]
44. Novak P, Li C, Shevchuk AI, Stepanyan R, Caldwell M, Hughes S, Smart TG, Gorelik J, Ostanin VP, Lab MJ, et al. Nanoscale Live-Cell Imaging Using Hopping Probe Ion Conductance Microscopy. *Nat Methods*. 2009; 6:279–281. [PubMed: 19252505]
45. Takahashi Y, Murakami Y, Nagamine K, Shiku H, Aoyagi S, Yasukawa T, Kanzaki M, Matsue T. Topographic Imaging of Convolved Surface of Live Cells by Scanning Ion Conductance Microscopy in a Standing Approach Mode. *Phys Chem Chem Phys*. 2010; 12:10012–10017. [PubMed: 20485766]
46. Adenle OA, Fitzgerald WJ. Simulating Scanning Ion Conductance Microscopy Using a Monte Carlo Approach. *Scanning*. 2006; 28:79–80.
47. Edwards MA, Williams CG, Whitworth AL, Unwin PR. Scanning Ion Conductance Microscopy: a Model for Experimentally Realistic Conditions and Image Interpretation. *Anal Chem*. 2009; 81:4482–4492. [PubMed: 19405486]
48. Nitz H, Kamp J, Fuchs H. A Combined Scanning Ion-Conductance and Shear-Force Microscope. *Probe Microsc*. 1998; 1:187–200.
49. Rheinlaender J, Schäffer TE. Image Formation, Resolution, and Height Measurement in Scanning Ion Conductance Microscopy. *J Appl Phys*. 2009; 105:094905.
50. Rheinlaender, J.; Schäffer, TE. Scanning Ion Conductance Microscopy. In: Kalinin, SV.; Gruverman, A., editors. *Scanning Probe Microscopy of Functional Materials: Nanoscale Imaging and Spectroscopy*. 1. Springer; New York, NY: 2011. p. 433–460.
51. Chen CC, Derylo MA, Baker LA. Measurement of Ion Currents through Porous Membranes with Scanning Ion Conductance Microscopy. *Anal Chem*. 2009; 81:4742–4751. [PubMed: 19435340]
52. Chen CC, Baker LA. Effects of Pipette Modulation and Imaging Distances on Ion Currents Measured with Scanning Ion Conductance Microscopy (SICM). *Analyst*. 2011; 136:90–97. [PubMed: 21103593]
53. Ebejer N, Schnipper M, Colburn AW, Edwards MA, Unwin PR. Localized High Resolution Electrochemistry and Multifunctional Imaging: Scanning Electrochemical Cell Microscopy. *Anal Chem*. 2010; 82:9141–9145. [PubMed: 20949925]
54. Lai SCS, Dudin PV, Macpherson JV, Unwin PR. Visualizing Zeptomole (Electro)Catalysis at Single Nanoparticles within an Ensemble. *J Am Chem Soc*. 2011; 133:10744–10747. [PubMed: 21671660]
55. Fleischer, RL.; Price, PB.; Walker, RM. *Nuclear Tracks in Solids*. University of California Press; Berkeley, CA: 1975.
56. Siwy Z, Gu Y, Spohr HA, Baur D, Wolf-Reber A, Spohr R, Apel P, Korchev YE. Rectification and Voltage Gating of Ion Currents in a Nanofabricated Pore. *Europhys Lett*. 2002; 60:349–355.
57. Davenport M, Rodriguez A, Shea KJ, Siwy ZS. Squeezing Ionic Liquids through Nanopores. *Nano Lett*. 2009; 9:2125–2128. [PubMed: 19354298]
58. Korchev YE, Milovanovic M, Bashford CL, Bennett DC, Sviderskaya EV, Vodyanoy I, Lab MJ. Specialized Scanning Ion-Conductance Microscope for Imaging of Living Cells. *J Microsc (Oxf)*. 1997; 188:17–23.

Figure 1.

Optical (a) and SEM (b, c) images of a representative single cylindrical pore membrane prepared. The large oval shape observed in the optical image (a) indicates the boundary of the epoxy painting, inside of which the membrane can be exposed to electrolyte from both sides. White dashed circles on (a, b) indicate the locations of the isolated nanopores on the membrane. The pore diameter of the isolated cylindrical pore (c) shown here was characterized as 542 nm.

Figure 2.

Schematic (a, b) of the relative positions of an SICM probe to a single pore membrane mounted on a perfusion cell. When the SICM probe is away from the pore center (a), the corresponding equivalent circuit (c) involves an access resistance, R_{acc} , generated from the probe-sample separation. In contrast, when the SICM is positioned over the pore center (b), another access resistance, R'_{acc} that results that includes the borders of the nanopore and the outer edge of the glass wall of the nanopipette is present in the equivalent circuit (d). PE: pipette electrode, RE: reference electrode and WE: working electrode.

Figure 3. Topography (a) and the corresponding current images (b, c, d) of an isolated single nanopore measured with the three-electrode SICM described here. Images correspond to (b) 0 V, (c) - 0.3 V and (d) + 0.3 V applied to the working electrode (WE). Scale bar: 1 μm .

Figure 4.

(a) Position-dependent current-voltage responses measured when the SICM probe was located over the pore center (■) and 8.5 μ m away from the pore center (●). (b) Simulated data calculated from proposed equivalent circuit models.

Figure 5.

(a) Vertical distribution of the current-voltage responses characterized with three-electrode SICM over the center of a single cylindrical nanopore. (b) Simulated data produced from the equivalent circuit model shown in Figure 2d.

Figure 6.

(a) Current-voltage responses measured with an SICM probe ($D_{ps} = 170$ nm) at different of lateral displacements from the pore center. (b) Line profiles of the current magnitude measured at various applied potentials reconstructed from current-voltage responses recorded at different lateral displacements.

Figure 7.

(a) Current-voltage responses recorded with an SICM probe for a single conical pore. When the SICM probe was located over the pore center, current rectification was observed (■). In contrast, the magnitude of the pipette current remained almost constant independent to the applied potential when the current-voltage response was measured 5 μm away from the pore center (●). (b) Changes in the absolute pipette current ($\Delta I = I_{\text{pipette, WE}} - I_{\text{pipette, WE}=0}$) recorded when the SICM probe was located over the pore center were plotted as a function of the potential applied to the working electrode.