Published in final edited form as:

Anal Chem. 2012 March 20; 84(6): 3003–3009. doi:10.1021/ac300257q.

Heterogeneity of Multiple-pore Membranes Investigated with Ion Conductance Microscopy

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

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

Abstract

Heterogeneous conductance of individual pores on a porous membrane was studied with a four-electrode scanning ion conductance microscope (SICM). Application of a potential difference across the membrane resulted in migration of ions through nanopores, where subsequent conductance changes were measured by a nanopipet positioned above the nanopore as a change in pipet current. Current responses of single-pore membrane and individual pores within a multi-pore membrane were examined and demonstrated variations in ion current rectification (ICR) ratios due to the small differences in pore geometries.

INTRODUCTION

In biological systems, methods to study and assess ion transport, such as patch-clamp techniques, have played key roles in understanding physiological and pathophysiological processes at a molecular level.^{1–6} A significant effort has been expended in further development of methods for measurement of ion transport in both biological and synthetic systems. Here, we describe a four-electrode scanning ion conductance microscope (SICM), which can measure characteristics of systems with heterogeneous conductance at high spatial resolution. Specifically, nanopore membranes with heterogeneous pores are examined, with unique conductance observed as a consequence of individual pore geometries.

The scanning ion conductance microscope (SICM) employs a glass nanopipet to scan the surface of a sample bathed in electrolyte.⁷ Distance between the SICM probe and the sample is monitored through the pipet ion current, which is used as feedback, to result in a non-contact imaging mode where vertical and lateral positions of the pipet are precisely controlled.^{7–12} SICM has been shown to operate well under physiological conditions to collect topographic and current information simultaneously.^{4,8,13–15} Membrane transport has been investigated previously with scanning probe microscopy, notably scanning electrochemical microscopy (SECM).^{16–24} We,^{25,26} and others,^{7,27,28} have previously described measurements of porous samples with SICM.

Recently, we have described a three-electrode configuration of SICM which enables the current-voltage responses of a single-nanopore to be examined.²⁹ We now extend this technique to measurement of multipore membranes. Here, the individual current-voltage response was examined for multiple conical pores in a membrane, where unique conductivities of each pore were observed. Summation of individual measurements of all pores in the membrane resulted in a current-voltage response that reflected the transport

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

properties of the membrane as a whole, verifying the accurate measurement of conductivities with this technique.

EXPERIMENTAL SECTION

Chemicals and materials

Deionized water (resistivity = 18 M Ω -cm) was obtained from a Milli-Q water purification system (Millipore Corp., Danvers, MA) for all solutions prepared. Sodium iodide (Mallinckrodt, Philipsburg, NJ) and sodium hypochlorite (10–15% active chlorine, Sigma-Aldrich, St. Louis, MO) were utilized to prepare nanoporous membranes from ion-tracked polyimide membranes (track density 10⁴ tracks/cm², thickness 25 μ m, it4ip, Belgium). Potassium chloride (Mallinckrodt, Philipsburg, NJ) solutions filtered by 0.22 μ m PVDF filter membrane (Millipore Corp., Danvers, MA) were utilized as electrolyte for ICM measurements. Water-resistive epoxy (Devcon, Riviera Beach, FL) was applied to isolate several nanopores from a membrane as described below. Nanopipets used in the study have an inner diameter 62 $\pm$ 28 nm and an outer diameter 258 $\pm$ 40 nm (Supporting information, Figure S1), and were filled with 0.1 M KCl.

Membrane preparation and characterization

Membranes with conical nanopores were prepared through an asymmetric track-etch process.^{30,31} Briefly, an ion-tracked polyimide membrane was placed between two halves of a U-tube cell. This assembly was then filled with sodium hypochlorite (etchant) on one side and sodium iodide (stopping solution) on the opposing side, and subsequently immersed in a 50 °C water bath for 98 min to obtain the desired pore sizes.

After the etching process, polyimide membranes with conical pores were mounted on a glass slide and examined with an inverted optical microscope to find a proper area for pore isolation. Water-resistive epoxy was painted onto the membrane face that possessed the large (base) diameter of the conical pores. With this process, the number of pores isolated could be controlled. Membranes with isolated pores were then mounted between two chambers of a horizontal perfusion cell, with the tip side of conical pores placed facing up.

Single- and multi- pore membranes were characterized by scanning electron microscopy (SEM, FEI Quanta-FEG) after ICM measurements. Membrane with a single-pore was prepared with pore diameters of 667 nm at the base side of the conical pore (Figure S2). Tip diameter was determined to be 89 nm as described in the supporting information. Optical and SEM images (base side) of the multi-pore membrane utilized in this study are shown in Figure 1. There are six pores in total on this membrane. Pores 1 and 2 are close to each other with a separation of $\sim$ 1.5 μ m, which cannot be resolved in this optical image. In Figure 1, the bright oval in the optical image is the boundary of the epoxy paint, and the central exposed area shown in the SEM image was clean and suitable for further investigations of nanopore conductance with the ICM probe. The same membrane was examined with SEM after ICM measurements to determine pore sizes. Base diameters for pores 1–6 ranged from 630–690 nm (Figure S3a–d, Table S1). Tip diameters for pores 1–5 determined from SEM images were ranged from 60–150 nm (Figure S3e–h, Table S1). The tip of pore 6 could not be located by SEM.

Instrumentation

A schematic of the four-electrode SICM system utilized in this study is shown in Figure 2. The SICM was operated in non-modulated and distance-modulated modes as has been described previously.^{7–9,11,14} A membrane with one or several conical pores was mounted between the upper and the lower chambers of a perfusion cell, filled with 0.1 M KCl. In this

four-electrode system, the pipette electrode (PE) was located in the upper chamber and held at a constant bias at a potential of +0.1 V with respect to the reference electrode (RE). A Ag/AgCl working electrode (WE) was introduced to the lower chamber of the cell to drive ions through the pores of the membrane. Potentials applied to the WE were controlled via a function generator (Agilent 33220A, Loveland, CO).

To prevent fluctuations in the potential of RE from passage of current across the multi-pore membrane, a counter electrode driver (CE driver), comprised of an amplifier circuit for a conventional potentiostat, was utilized. Both the RE and a platinum counter electrode (CE) were located in the upper chamber and connected to separate input channels of the CE driver. Here, the majority of the cross-membrane current generated by the potential differences between RE and WE was driven by the counter electrode (CE) as in a conventional potentiostat. Both the function generator, which controlled the potential applied to WE and the CE driver were grounded to a faraday cage, which enclosed the microscope.

Before ICM measurements for each nanopore, an SICM current image was collected in distance-modulated feedback mode with a potential (e.g. +0.5 V or -0.5 V) applied to WE. The pipet current displayed significant changes when the pipet scanned over a pore. These current images were utilized to determine the precise positions of individual pores. While the current images can provide conductance information for each pore simultaneously within a single image, the magnitude of currents obtained with distance-modulated feedback mode can be influenced by convection effects introduced by pipet modulation.^{25,26} Thus, static measurements of the pipet currents with non-modulated mode were performed instead.

With pore positions determined from the current images, the ICM probe (nanopipet) was positioned over the center of a selected pore with a probe-sample distance (D_{ps}) maintained at 150–180 nm, as determined experimentally. The potential applied to WE was then swept from -1.0 V to +1.0 V and changes in the pipet current were recorded as a function of the potential applied to WE. The same measurement was performed for each individual pore of the multi-pore membrane to determine the conductance properties of each individual pore. Topographic and current images of the single-pore and multi-pore membranes, as well as the pipet current measurements were acquired with a ScanIC scanning ion conductance microscope (ionscope, Ltd., London, UK) combined with an Axopatch 200B current amplifier (Molecular Devices, Union City, CA).

RESULTS AND DISCUSSION

ICM signals recorded for single conical pore membrane

Static ICM current-voltage measurements were performed first on a membrane that possessed a single conical pore (Figure S2a), as described previously.²⁹ Here, when the ICM probe was located over the center of a nanopore with a probe-sample distance (D_{ps}) at the order of nm, an access resistance of ~1 M Ω exists (Figure 2).

For all ICM measurements, a potential of +0.1 V was applied to the PE ($V_{PE} = +0.1$ V) to result in a pipet current of ~0.6–1.0 nA, when the WE was held at 0 V. The potential difference between the PE and the RE (V_{PE}) was divided by the potential drop at the pipet resistance, and by the potential drop at the access resistance, which is the small gap between pipet tip and sample.

The access resistance plays a key role in ICM experiments, as it involves pathways of ion current between the PE and the RE, as well as between the WE and the RE (Figure 2). Here, the access resistance works as a voltage divider. Since the potential applied to the PE is

constant at +0.1 V, when the potential applied on the WE changes (V_{WE}), variations in the potential drop at the access resistance occurs and affects the potential drop on the pipet. Thus, the access resistance contributes to changes in the potential drop across the ICM probe as a function of the potential applied to the WE. Changes in the potential drop on the pipet eventually results in changes of the pipet current.

Based on the equivalent circuit diagrams (Figure S4) for the experimental setup shown in Figure 2, relationships of the resultant currents to potentials applied to the WE can be described by equations 1 and 2 (details of derivation are in the supporting information). Here, V_{WE} is the potential applied on the WE, which is swept from -1 V to +1 V. R_{pipet} and R_{acc} represent the pipet and the access resistances, respectively, while $R_{feature}$ indicates the resistance associated with the local feature under study with the ICM probe (pipet). In this study, pipet and access resistances were measured to be ca. 90 M Ω and 1 M Ω , respectively. Here, $I_{feature}$ is the ion current through the feature under study while I_{pipet} is the ion current through the pipet, which is the ICM signal recorded.

$$I_{pipet} = \frac{-V_{WE}R_{acc} + 0.1R_{acc} + 0.1R_{feature}}{R_{feature}R_{pipet} + R_{feature}R_{acc} + R_{acc}R_{pipet}} \quad (1)$$

$$I_{feature} = \frac{V_{WE}R_{pipet} + V_{WE}R_{acc} - 0.1R_{acc}}{R_{feature}R_{pipet} + R_{feature}R_{acc} + R_{acc}R_{pipet}} \quad (2)$$

When the pipet is located over a pore center, $R_{feature}$ (Equations 1 and 2) can be replaced by the pore resistance (R_{pore}), which is on the order of hundreds of M Ω for the conical pores studied here. In contrast, when the pipet is located over the membrane (away from the center of a nanopore), $R_{feature}$, which indicates the resistance of the local feature under study, is infinite because the membrane utilized here is an insulator. Consequently, the magnitude of I_{pipet} can be represented as $0.1/(R_{pipet} + R_{acc})$, independent of the applied WE potential. In other words, measured pipet currents are not affected by the WE potentials when the pipet is placed over features located far from ion transport pathways. The pipet current measured when the pipet was located over the pore center and over the membrane of a single-pore membrane are plotted with the potential applied on the WE in Figure 3a (red line and black line), which agree with the current behavior predicted by the proposed equivalent circuits.

ICM measurements reported here describe ion transport properties of features of interest (i.e. nanopores in this study). To achieve this goal, we attempt to derive a relationship to correlate the measured ICM signals (I_{pipet} in Equation 1) with the transport properties of the feature (nanopore) investigated ($I_{feature}$ in Equation 2, which can be represented as I_{pore} here). Consequently, instead of the absolute pipet current measured (I_{pipet}), changes in the pipet current (ΔI_{ICM} in Equation 3) with respect to the pipet current measured at 0 V of the WE potential were utilized. Equation 5 shows a simple relationship obtained to describe the transport properties of the nanopore (ΔI_{pore} in Equation 4) with changes in the pipet current measured (ΔI_{ICM}).

$$\Delta I_{ICM} = I_{pipet, WE(V)} - I_{pipet, WE(0V)} = \frac{-V_{WE} \cdot R_{acc}}{R_{pore}R_{pipet} + R_{pore}R_{acc} + R_{acc}R_{pipet}} \quad (3)$$

$$\Delta I_{pore} = I_{pore, WE(V)} - I_{pore, WE(0V)} = \frac{V_{WE} \cdot (R_{pipet} + R_{acc})}{R_{pore} R_{pipet} + R_{pore} R_{acc} + R_{acc} R_{pipet}} \quad (4)$$

$$\frac{\Delta I_{ICM}}{\Delta I_{pore}} = -\frac{R_{acc}}{R_{pipet} + R_{acc}} \rightarrow -\Delta I_{ICM} = \frac{R_{acc}}{R_{pipet} + R_{acc}} \cdot \Delta I_{pore} \quad (5)$$

$$-\Delta I_{ICM} \propto \Delta I_{pore} \propto V_{WE} \quad (6)$$

Equation 5 describes the change in current of the ICM probe, which is proportional to the negative of the change in current of the pore investigated. Therefore, $-\Delta I_{ICM}$ instead of ΔI_{ICM} was plotted as a function of the potential on the WE to describe changes in the pore current due to the applied WE potential (Equation 6). In addition, the description of ICM measurements with the relationship between $-\Delta I_{ICM}$ and applied WE potentials facilitates comparison with conventional current-voltage measurements (e.g. made with macroscopic two-electrode systems) as shown in Figure 3b.

For conical pores in a polyimide membrane prepared here, the surface is negatively charged at neutral pH, and higher currents (lower pore resistance/higher pore conductivity) are observed when a negative potential is applied (WE on the base side of the membrane) than when the corresponding positive potentials are applied. In general, enhanced current rectification can also be observed when the double layer on the inner surface of a conical pore is comparable to the tip diameter of the conical pore.^{32,33} This non-linear resistance of a conical pore leads to non-linear changes in absolute pipet currents measured with the ICM probe as a function of the WE potentials applied (Figure 3a, red line). Therefore, a rectified current-voltage relationship between the $-\Delta I_{ICM}$ and the WE potentials can be obtained, as shown in Figure 3b. By examining the $(-\Delta I_{ICM})$ -voltage responses recorded with an ICM probe, the ion current rectification ratio (ICR, $|\Delta I_{-V}/\Delta I_{+V}|$) which implies ion transport properties of a conical pore can be characterized.³⁴⁻³⁷

The single conical pore membrane was investigated by both ICM recordings and macroscopic conductivity measurements. The ICR ratios determined from ICM measurements ($|\Delta I_{-0.6V}/\Delta I_{+0.6V}|$, Figure 3b) and from macroscopic measurements ($|I_{-0.6V}/I_{+0.6V}|$, inset of Figure 3b) agree well for single-pore membrane. This result demonstrates that ICM measurements made here are able to correctly represent ion transport properties of a nanopore. In addition, another single pore isolated from the same etched membrane displayed significant difference in current rectification responses (Figure S5). From ICM measurements, the conical pore utilized to obtain Figure 3b with an ICR ratio of 1.7 exhibits much less rectification than the other conical pore whose ICR ratio is 22.8 (Figure S5b). This result implies that individual nanopores of a multi-pore membrane exhibit heterogeneous ion transport properties.

Heterogeneous current-voltage responses for pores on a multi-pore membrane

To examine heterogeneous ion transport properties of individual pores on the same membrane, a membrane with six pores was chosen. Changes of the pipet current (ΔI_{ICM}) measured with the ICM probe were recorded as a function of the potential applied to the working electrode (WE). Figure 4a shows the relationships of $-\Delta I_{ICM}$ and the applied WE potential of individual pores on the six-pore membrane measured with the ICM probe over the center of individual pores. ICR ratios ($|\Delta I_{-1V}/\Delta I_{+1V}|$) characterized from ICM measurements for pores 1–6 are 1.1, 1.3, 1.2, 1.4, 66.0 and 5.3, respectively.

For the six pores studied here, significant differences in the current-voltage relationship were measured. For pores 1–4, ICR ratios close to 1 were observed. In contrast, pores 5 and 6 display strongly rectified current-voltage relationships, with ICR measurements of 66.0 and 5.3, respectively. Electron micrographs of pores 1–4 indicated larger overall pore tip diameters (98–104 nm), when compared to pore 5 (66 nm). Pore 6 could not be located by SEM. Previous work has demonstrated the nature of pore geometry, both cone angle and pore diameter on ICR phenomenon.^{33,38} Here, smaller pores rectify more, in agreement with these reports. Further, the surface charge density is known to also influence the extent of ICR.^{39,40} For pores here, it is possible, in addition to differences in geometry, surface charge densities may also differ as a consequence of film inhomogeneity. The dramatic ICR ratio observed for pore 5 (ICR ratio = 66.0) suggests a high surface charge density, extreme pore geometry (or both). Variations in ICR ratios suggest that there is heterogeneity among pores on track-etched membranes as prepared here. With conventional macroscopic measurements, the current-voltage response recorded indicates the average transport properties of multiple pores on the membrane but ignores the individual attributes of each single pore. However, with ICM measurements, the heterogeneous current-voltage responses for individual pores from the same etched membrane can be obtained.

In addition, the relationships of $-\Delta I_{\text{ICM}}$ to the applied WE potential measured by ICM for 6 individual pores of a multi-pore membrane can be summed to produce a composite ICM signal shown as the solid red line in Figure 4b. This composite ICM signal with an ICR ratio ($|\Delta I_{-1} \text{ V} / \Delta I_{+1} \text{ V}|$) of 2.0 agrees well with the average current-voltage response of this six-pore membrane determined from a traditional macroscopic two-electrode measurement (dotted black line, in Figure 4b, ICR ratio ($|\Delta I_{-1} \text{ V} / \Delta I_{+1} \text{ V}|$) = 1.9). This finding further demonstrates that ICM signals recorded for each pore on a multi-pore membrane not only indicate the ion transport properties of individual pores but can also be utilized to produce a composite ICM signal that is representative of the ion transport properties of the membrane as a whole.

Position-dependent transitions of the ICM signals recorded for two pores with distinct ion transport properties

With the current-voltage responses measured with an ICM probe for isolated single-pore membrane (Figure 3a), it is clear that the ICM signals recorded are affected by the relative position of the ICM probe with respect to the center of a pore. This position-dependent characteristic implies that the ICM signals detected reflect ion transport properties of individual nanopores under study independent of other nanostructures involved in the same system. To confirm this assumption, ICM signals measured as a function of lateral positions were investigated.

With SICM operated under distance-modulated mode, scanning current images for pores 4 and 5 with both -0.5 V and $+0.5 \text{ V}$ applied to WE were recorded as shown in Figure 5a and 5b, respectively. With Figure 5a, a separation between pores 4 and 5 of $19 \mu\text{m}$ was observed. At this potential (-0.5 V), the magnitude of the ion currents measured for pores 4 and 5 exhibit similar intensity. However, the width of the current profiles resolved for pore 4 (tip diameter 103 nm) was much larger than that of pore 5 (tip diameter 66 nm) due to the difference in tip sizes of these two conical pores. In contrast, when a WE potential of $+0.5 \text{ V}$ was applied, apparent differences in the current intensity measured for pores 4 and 5 were observed as shown in Figure 5b. Here, no current intensity was detected for pore 5 when the WE electrode was held at $+0.5 \text{ V}$, while an ion current with an absolute magnitude of 11 pA was observed for pore 4.

From pore locations determined from current images recorded in scanning mode, ICM signals measured at fixed positions with a probe-sample distance of 180 nm along a line that

connects the centers of pores 4 and 5 were recorded. The ICM probe was brought to the center of pore 4 and the $-\Delta I_{\text{ICM}}$ as a function of applied WE potentials was recorded as shown in Figure 5c. From Figure 5c, no significant rectification was observed for pore 4. When the ICM probe was located at the midpoint between pores 4 and 5 (9.5 μm away from both pores centers), changes in pipet current (ΔI_{ICM}) were essentially independent to the potential applied to WE (Figure 5d). Consistent with what was obtained for single-pore membranes, this observation verifies that changes in pipet current (ΔI_{ICM}) measured are position dependent and are not affected by the transport properties of pores far from the ICM probe, even in the case of the multi-pore membrane. Figure 5e was obtained by positioning the ICM probe over the center of pore 5, where significant rectification was observed. This result further confirms the assumption that ion transport properties are quite different between pore 4 and pore 5 as observed with the current images shown in Figure 5a and 5b.

In addition, if the values of $-\Delta I_{\text{ICM}}$ recorded at a WE potential of -0.5 V are considered (Figure 5c and 5e), similar magnitudes of $-\Delta I_{\text{ICM}}$ (about -40 pA) for both pores 4 and 5 are observed. This agrees well with Figure 5a where the peak values of the imaged current profiles for pores 4 and 5 are similar ($-\Delta I_{\text{ICM}} \approx -14\text{ pA}$). However, when $+0.5\text{ V}$ was applied to the WE, $-\Delta I_{\text{ICM}}$ measured for pore 4 ($-\Delta I_{\text{ICM}} \approx 26\text{ pA}$) was much larger than that of pore 5 ($-\Delta I_{\text{ICM}} \approx 0$) in agreement with the case of Figure 5b. From Figure 5, the ICM signals recorded for individual pores of a multi-pore membrane are shown to correctly reveal the ion transport properties of the pore under study independent of other system conductivities.

Resolution of ICM measurements

To investigate resolution of this four-electrode ICM system on ion transport measurements, two pores separated at a small distance were examined. Figure 6a is the SICM current image measured with WE held at -0.5 V of pores 1 and 2 on the membrane utilized here. The separation between these two pores was characterized as $1.5\text{ }\mu\text{m}$ from the SEM image shown in Figure 6b (tip side). From the SICM current image shown in Figure 6a, current profiles measured are observed to be larger in width than the actual pore tip sizes. This phenomenon has been found commonly in all types of scanning probe microscopy due to convolution of the scanning probe and the surface feature under study. The convolution effect is exacerbated when the probe size is larger than features of interest, as encountered here. (SICM probes with an average outer diameter of 250 nm , pore 1 with 146 nm in diameter, pore 2 with 98 nm in diameter).

Current-voltage responses recorded with an ICM probe at fixed positions were investigated. Figure 6c illustrates the ICM signals recorded by plotting $-\Delta I_{\text{ICM}}$ as a function of the applied WE potential at five positions along a line through the centers of pores 1 and 2 (Figure 6a). At positions 2 and 4, changes in pipet current (ΔI_{ICM}) measured over the centers of pores 1 and 2, respectively, were found to be strongly dependent on the potential applied to the WE. The current-voltage relationships measured at the centers of pores 1 and 2 displayed different slopes (Figure 6c, positions 2 and 4) which can be explained from the pore sizes determined from SEM, where pore 1 is larger than pore 2 in both tip and base sizes, and thus has a higher conductivity.

In addition, the ICM signals measured at position 1 (750 nm away from pore 1), position 3 (750 nm away from both pore 1 and pore 2) and position 5 (750 nm away from pore 2) show similar slopes. These slopes are not zero, however, as shown in Figure 5d (measured at the middle point of two pores which is $19\text{ }\mu\text{m}$ away from each other). These non-zero current-voltage responses in the vicinity of the pores imply that ICM measurements can be influenced by other pathways of conductivity that are at close distances. However, in the case here the differences in the slopes of the ICM signals recorded over pore centers (Figure

6c, positions 2 and 4) and away from pore centers (Figure 6c, positions 1, 3 and 5) are significant enough to identify if an ICM signal was measured over an active ion transport pathway. Furthermore, from Figure 6c, ion transport properties of two pores which are separated by a very small distance (such as the 1.5 μm encountered here) can be obtained.

CONCLUSION

In this study, a modified scanning ion conductance microscope (SICM) with four electrodes was established to study heterogeneous ion conductance of individual pores of a multi-pore membrane. By locating the ICM probe over each pore center and measuring pipet current changes as a function of the potential applied across the membrane, each pore was investigated individually. The combined ICM signals of each pore were shown to exhibit the same ion current rectification ratio as recorded macroscopic measurements. The ability of ICM to distinguish structures with different conductance and at a distance $\leq 1.5 \mu\text{m}$ was demonstrated. Conductance measurements with SICM taken in this manner benefit from the robust control of vertical and lateral probe positions, as well as the capability to function in physiological conditions, to provide a promising platform to study ion transport pathways in more complex polymeric or biological structures. Future improvements in that make use of nanopipets with smaller tip diameters or closer probe-sample separations are expected to increase the resolution of this technique.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded 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 thank Mr. Andy Alexander and Mr. John Poehlman for the technical support. We also appreciate valuable suggestions of pore isolation from Mr. Joseph Basore.

LITERATURE CITED

1. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. *Pfluegers Arch.* 1981; 391:85–100. [PubMed: 6270629]
2. Pusch M, Neher E. *Pfluegers Arch.* 1988; 411:204–211. [PubMed: 2451806]
3. Gu YC, Gorelik J, Spohr HA, Shevchuk A, Lab MJ, Harding SE, Vodyanoy I, Klenerman D, Korchev YE. *FASEB J.* 2002; 16:748–750. [PubMed: 11923226]
4. Korchev YE, Negulyaev YA, Edwards CRW, Vodyanoy I, Lab MJ. *Nat Cell Biol.* 2000; 2:616–619. [PubMed: 10980702]
5. Fenwick EM, Marty A, Neher E. *J Physiol.* 1982; 331:577–597. [PubMed: 6296371]
6. Dale TJ, Townsend C, Hollands EC, Trezise DJ. *Mol BioSyst.* 2007; 3:714–722. [PubMed: 17882333]
7. Hansma PK, Drake B, Marti O, Gould SAC, Prater CB. *Science.* 1989; 243:641–643. [PubMed: 2464851]
8. Korchev YE, Bashford CL, Milovanovic M, Vodyanoy I, Lab MJ. *Biophys J.* 1997; 73:653–658. [PubMed: 9251784]
9. Shevchuk AI, Gorelik J, Harding SE, Lab MJ, Klenerman D, Korchev YE. *Biophys J.* 2001; 81:1759–1764. [PubMed: 11509385]
10. Mann SA, Hoffmann G, Hengstenberg A, Schuhmann W, Dietzel ID. *J Neurosci Methods.* 2002; 116:113–117. [PubMed: 12044660]

11. Novak P, Li C, Shevchuk AI, Stepanyan R, Caldwell M, Hughes S, Smart TG, Gorelik J, Ostanin VP, Lab MJ, Moss GWJ, Frolenkov GI, Klenerman D, Korchev YE. *Nat Methods*. 2009; 6:279–281. [PubMed: 19252505]
12. Takahashi Y, Murakami Y, Nagamine K, Shiku H, Aoyagi S, Yasukawa T, Kanzaki M, Matsue T. *Phys Chem Chem Phys*. 2010; 12:10012–10017. [PubMed: 20485766]
13. Schäffer, TE.; Anczykowski, B.; Fuchs, H. Scanning Ion Conductance Microscopy. In: Bhushan, B.; Fuchs, H., editors. *Applied Scanning Probe Methods II*. Springer-Verlag; Berlin, Heidelberg: 2006. p. 91-119.
14. Korchev YE, Milovanovic M, Bashford CL, Bennett DC, Sviderskaya EV, Vodyanoy I, Lab MJ. *J Microsc (Oxf)*. 1997; 188:17–23.
15. Miragoli M, Moshkov A, Novak P, Shevchuk A, Nikolaev VO, El-Hamamsy I, Potter CMF, Wright P, Kadir S, Lyon AR, Mitchell JA, Chester AH, Klenerman D, Lab MJ, Korchev YE, Harding SE, Gorelik J. *J Roy Soc Interface*. 2011; 8:913–925. [PubMed: 21325316]
16. Bath BD, Lee RD, White HS, Scott ER. *Anal Chem*. 1998; 70:1047–1058.
17. Bath BD, White HS, Scott ER. *Anal Chem*. 2000; 72:433–442. [PubMed: 10695125]
18. Uitto OD, White HS. *Anal Chem*. 2000; 73:533–539. [PubMed: 11217758]
19. McKelvey K, Snowden ME, Peruffo M, Unwin PR. *Anal Chem*. 2011; 83:6447–6454. [PubMed: 21776952]
20. Ervin EN, White HS, Baker LA, Martin CR. *Anal Chem*. 2006; 78:6535–6541. [PubMed: 16970331]
21. Ervin EN, White HS, Baker LA. *Anal Chem*. 2005; 77:5564–5569. [PubMed: 16131066]
22. Gardner CE, Unwin PR, Macpherson JV. *Electrochem Commun*. 2005; 7:612–618.
23. Uitto OD, White HS, Aoki K. *Anal Chem*. 2002; 74:4577–4582. [PubMed: 12236372]
24. Ebejer N, Schnippering M, Colburn AW, Edwards MA, Unwin PR. *Anal Chem*. 2010; 82:9141–9145. [PubMed: 20949925]
25. Chen CC, Baker LA. *Analyst*. 2011; 136:90–97. [PubMed: 21103593]
26. Chen CC, Derylo MA, Baker LA. *Anal Chem*. 2009; 81:4742–4751. [PubMed: 19435340]
27. Proksch R, Lal R, Hansma PK, Morse D, Stucky G. *Biophys J*. 1996; 71:2155–2157. [PubMed: 8889191]
28. Siwy Z, Gu Y, Spohr HA, Baur D, Wolf-Reber A, Spohr R, Apel P, Korchev YE. *Europhys Lett*. 2002; 60:349–355.
29. Chen CC, Zhou Y, Baker LA. *ACS Nano*. 2011; 5:8404–8411. [PubMed: 21923184]
30. Bean, CP.; DeSorbo, W. Porous Bodies and Method of Making. US Patent. 3,770,532. November 6. 1973
31. Fleischer, RL.; Price, PB.; Walker, RM. *Nuclear Tracks in Solids Principles and Applications*. Univ. of California Press; Berkeley: 1975.
32. Siwy Z, Heins E, Harrell CC, Kohli P, Martin CR. *J Am Chem Soc*. 2004; 126:10850–10851. [PubMed: 15339163]
33. Kubeil C, Bund A. *J Phys Chem C*. 2011; 115:7866–7873.
34. Siwy ZS. *Adv Funct Mater*. 2006; 16:735–746.
35. Ai Y, Zhang M, Joo SW, Cheney MA, Qian S. *J Phys Chem C*. 2010; 114:3883–3890.
36. Lan WJ, Holden DA, White HS. *J Am Chem Soc*. 2011; 133:13300–13303. [PubMed: 21800889]
37. White HS, Bund A. *Langmuir*. 2008; 24:2212–2218. [PubMed: 18225931]
38. Harrell CC, Siwy ZS, Martin CR. *Small*. 2006; 2:194–198. [PubMed: 17193019]
39. Wei C, Bard AJ, Feldberg SW. *Anal Chem*. 1997; 69:4627–4633.
40. Ali M, Schiedt B, Healy K, Neumann R, Ensinger W. *Nanotechnology*. 2008; 19:085713. [PubMed: 21730744]

Figure 1.

Optical (a) and SEM (b) images (base side) of the multi-pore membrane used in the study. The oval shapes are the boundaries of epoxy paint inside of which the tip side of six conical pores are exposed for ICM measurements. Pores are numbered according to the positions on the membrane (a), and pores 1 and 2 are close to each other which can't be resolved in the magnification used here. White dashed circles in (b) indicate the positions of nanopores.

Figure 2. Schematic of the modified four-electrode SICM. A multi-pore conical membrane is mounted in a perfusion cell filled with electrolyte (0.1 M KCl in both chambers). A counter electrode driver (CE driver) is utilized to maintain a stable potential on reference electrode (RE) (0 V). Potential applied on working electrode (WE) is controlled by a function generator. The ICM probe (nanopipet) is positioned over a pore center to measure current changes induced by sweeping the working electrode potential.

Figure 3.

(a) Changes in absolute pipet current of a representative single-pore membrane were plotted as a function of the potential applied to the working electrode (WE), which was swept from -1.0 V to $+1.0$ V. Measurements were performed when the pipet was located over the pore center (red line) and away from the pore ($5.9\ \mu\text{m}$) on the membrane (black line). (b) $-\Delta I_{ICM}$ ($\Delta I_{ICM} = I_{\text{pipet, WE}(V)} - I_{\text{pipet, WE}(0\text{ V})}$) was plotted with the potential on the WE to facilitate comparison with current-voltage responses measured with macroscopic two-electrode method (inset), which present similar trend in current rectification.

Figure 4.

(a) Current-voltage responses measured with ICM probe over each pore center with a probe-sample distance (D_{ps}) of 180 nm. (b) Composite current-voltage response of all pores measured with ICM and current-voltage responses of the membrane measured with conventional macroscopic two-electrode method.

Figure 5.

SICM current images of pores 4 and 5 at (a) -0.5 V and (b) $+0.5$ V. (c–e) Current-voltage responses for three pipet positions indicated in (a). (c) A nearly linear current-voltage relationship was observed when the pipet was positioned over pore 4. (d) When the pipet was located over the membrane away from a pore, no current response was detected. (e) A strongly rectified current response was measured with the pipet positioned over the center of pore 5.

Figure 6.

(a) SICM current image at -0.5 V applied to the WE, and (b) SEM image (tip side) of pores 1 and 2. (c) ICM measurements performed at five positions indicated in (a). Higher current responses were detected over a pore center. When the pipet was moved away from a pore center, the slope of ICM signals decreased, which differentiated the signal recorded over the pore center from those measured above other features on the membrane.