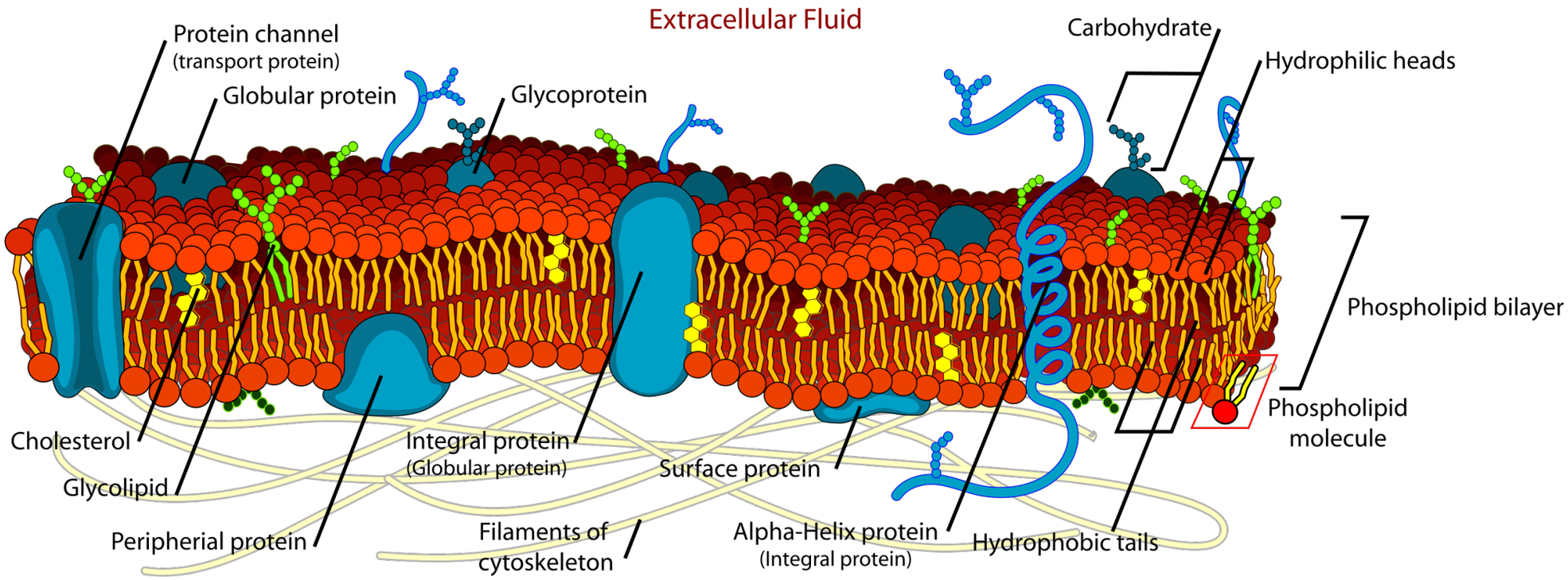
Cell Membrane Structure

fluid mosaic membrane model



The constituents of cell membranes include many different kinds of lipids, proteins, & other molecules (e.g., cholesterol). A fluid mosaic model for membranes was suggested by Singer & Nicholson (1972. Science **175**, 720), which significantly refined earlier membrane models posited by Gorter & Grendel (1925. *J Exp Medicine* **41,** 439), Danielli & Davson (1935. *J Cell and Comp Physiol* **5**, 495), and others.

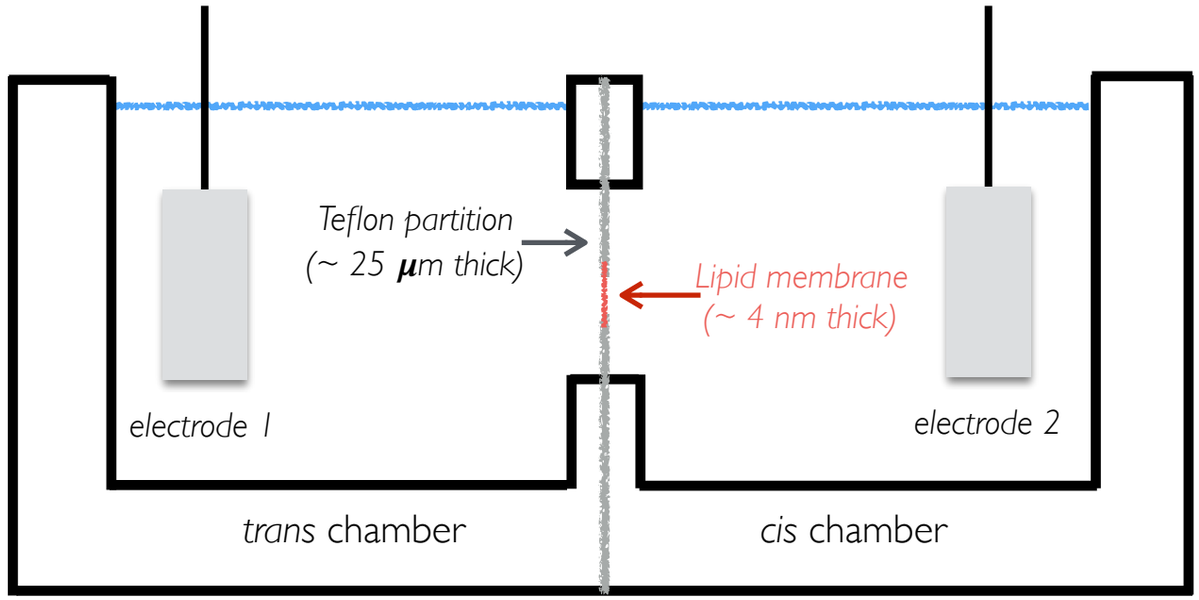
Lipids are comprised of hydrophobic hydrocarbon tails and polar head groups. These allow the lipid portion of the membrane to spontaneously self assemble into a bilayer structure in the presence of water. The fatty acid tails have a low dielectric constant ($\varepsilon \approx 2$) that resist the flow of ions across the lipid portion of the membrane (Parsegian 1969. *Nature* **221**, 844).

Charges species are transported across the membranes by ion channels, pumps, and other processes (e.g., exocytosis and pinocytosis).

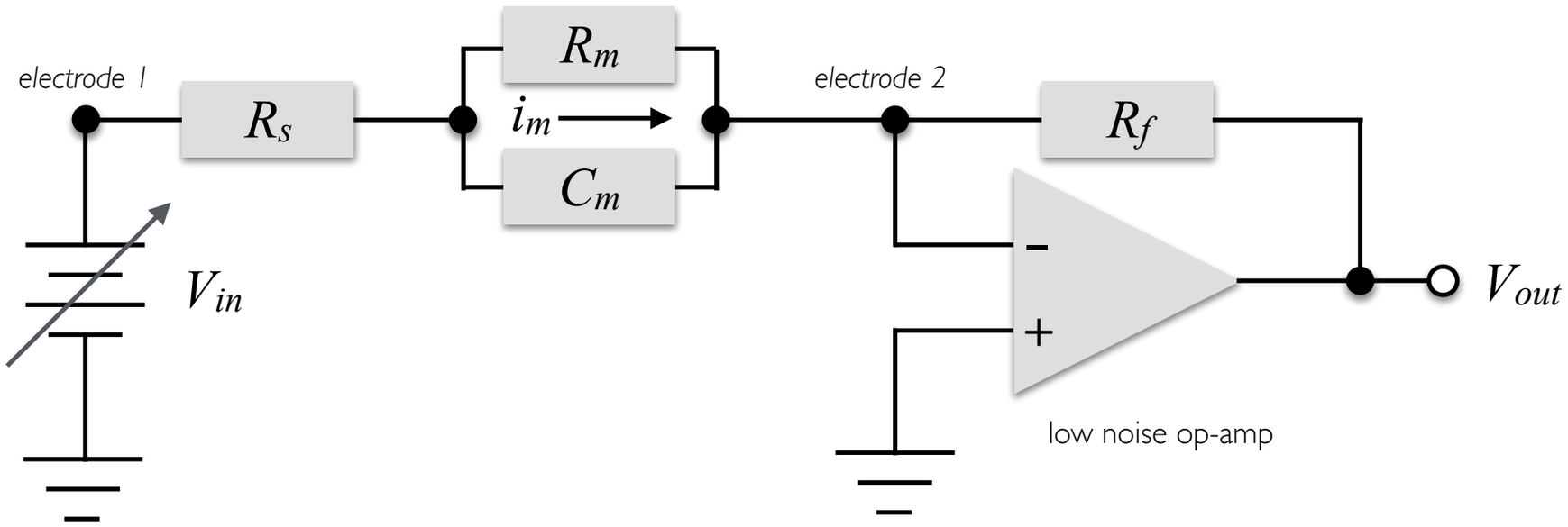
Lipid bilayer membranes, & ion channels reconstituted into them, have been studied using an artificial model system:
Black Lipid Membranes (BLMs)

BLM Measurement

basic principles



Teflon lipid bilayer chamber



Resistive Feedback

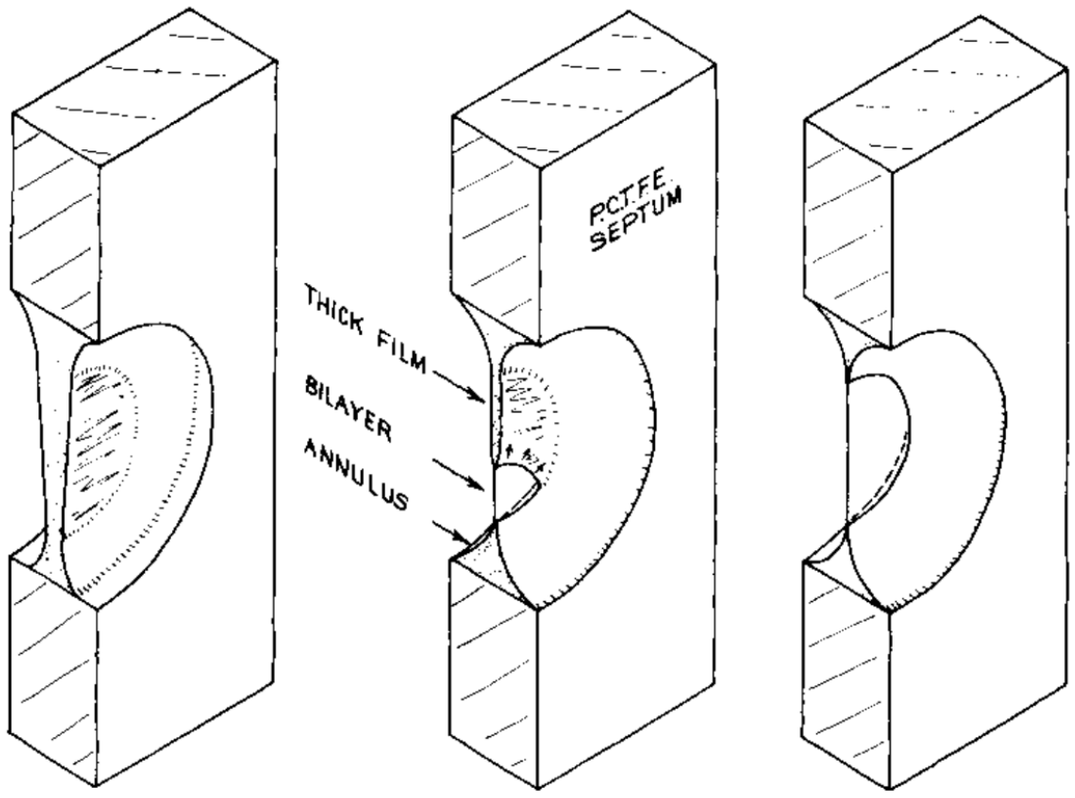
$$|R_m| = R_f \times V_{in}/V_{out}$$

where R_m & R_f are the membrane & feedback resistance values, respectively.

We apply V_{in} and the op-amp outputs V_{out} through R_f via negative feedback
to make the potential of the op-amp's inverting input equal to that of the non-inverting input.

BLM Painting Method

Mueller, Rudin, Tien, & Westcott. 1962. *Science* **194**, 979



White, S. Chapter 1, *in* Ion Channel Reconstitution. (C. Miller, editor)

Lipid membrane mimics are made from purified lipids or cellular lipid extracts on a small hole (diameter < 200 μm in a highly insulating thin film (e.g., Teflon). They are formed either by "painting" lipid dissolved in a solvent (e.g., decane) onto the hole pretreated with lipid in pentane (the method described in Mueller, et al. 1962) or using the "solvent-free" Montal-Mueller method (*described later*). The initial thickness of the film is initially ≈ the wavelength of visible light and colored interference fringes can be observed. When the membrane thins, the reflected fringes disappear and the transparent membrane looks black (because of the shadow behind it).

embryos raised at 25°, 20°, 18°, 15° and 10° C. respectively. As to myosin formation, the first detectable trace was found 108 hr. after fertilization in embryos raised at 25° C., 168 hr. when raised at 20° C., 180 hr. at 18° C., then again 168 hr. at 15° C. and 132 hr. at 10° C., as shown in Fig. 1. Thus, actin formation in early embryos was markedly suppressed with dropping temperature under the above experimental conditions. On the contrary, myosin formation was most strikingly suppressed at 18° C. and from thereon was clearly promoted independently of falling or rising temperature.

This finding is unexpected, but the result is noteworthy, because the temperature of the natural breeding season of *Triturus* is about 18° C. We may imagine that many metabolic pass-ways are in competition for the materials that are required to keep a balance necessary for the growth of the embryo. For example, there may be a metabolite present which at the optimum temperature for growth appropriates the materials necessary for myosin formation.

The order of actin and myosin formation in embryos raised at 10° C. is opposite to that found in natural development. This result furnishes further evidence of the independence of actin and myosin formation one from the other[2,4].

This work was supported by a grant-in-aid for fundamental scientific research (No. 710267) of the Ministry of Education in Japan.

YOSHITO OGAWA

National Institute of Genetics,
Misima, Japan.

[1] Ogawa, Y., *Nature*, **182**, 1312 (1958).
[2] Ogawa, Y., *Nature*, **186**, 77 (1960).
[3] Szent-Györgyi, A. G., *Chemistry of Muscular Contraction* (Academic Press, New York, 1951).
[4] Ogawa, Y., *Med. and Biol.*, **58**, 185 (1961) (in Japanese).

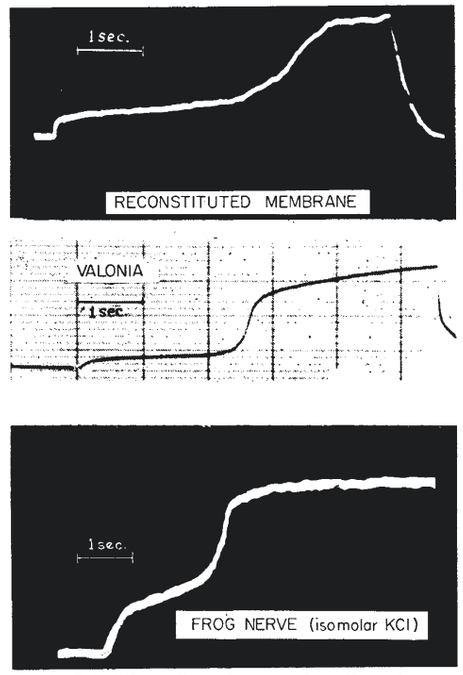


Fig. 1. Potential recordings of responses to applied rectangular currents. The membrane electromotance is very likely zero or close to zero in all three cases. Upper two are trans-membrane recordings, bottom record is proportional to trans-membrane potential of frog single node in 0·1 *N* potassium chloride. (Middle figure reprinted by permission from Blinks, L. R., *J. Gen. Physiol.*, **19**, 633; 1936)

PHYSIOLOGY

Reconstitution of Cell Membrane Structure *in vitro* and its Transformation into an Excitable System

THE formation of single, stable bimolecular lipid and proteolipid[1] membranes up to 10 mm.² in area has been accomplished routinely in 0·1 *M* saline solution by methods analogous to the formation of Hooke-Newton 'secondary black' in air soap films[2–4]. By forming such a membrane between two compartments filled with saline its transverse electrical properties can be measured, and controlled chemical investigations can be undertaken.

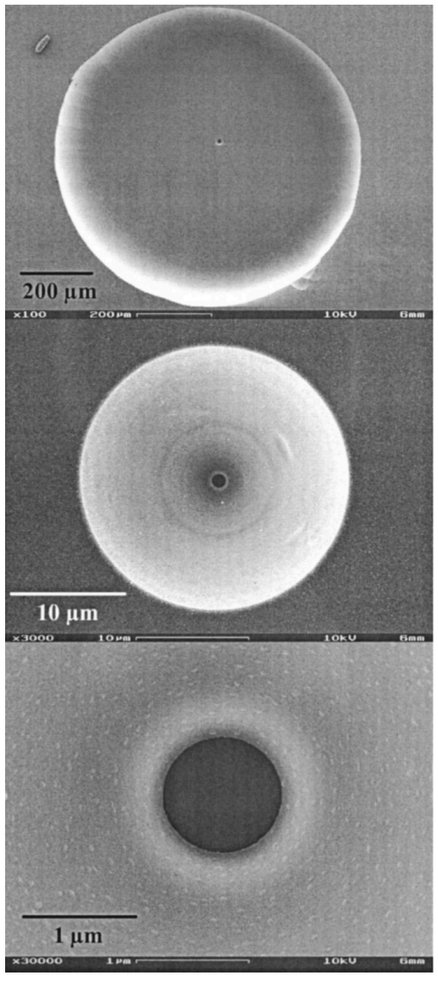
This experimental structure, invisible in transmitted light, appears as a faint grey sheen in reflected light at a large angle of incidence. It is grossly manipulable, resilient, self-sealing to puncture, liquid in the plane of the bilayer, stains with osmium tetroxide and is 60–90 Å. thick under the electron microscope. Electrical capacity is about 1μ F./cm.². Resistance is usually 10^7 but often greater than 10^8 ohm cm.² and is constant up to dieletric breakdown at 0·15–0·20 V. ($2{\cdot}5 \times 10^5$ V./cm.), whereupon the membrane shatters. It also breaks below *p*H 5·0 and above *p*H 9·0. In ionic gradients the unmodified membrane shows weak electrical polarization and poor ionic selectivity.

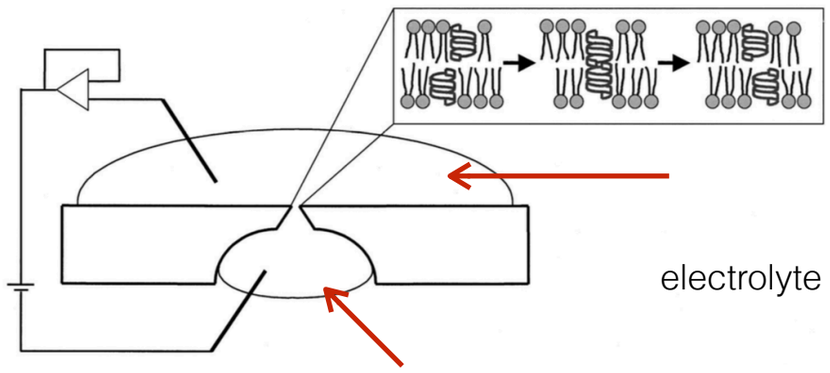
However, this inert structural barrier has been successfully modified by a variety of water-soluble macromolecules which spontaneously adsorb to it when added to the environmental solution. Some proteins adsorb but do not lower its resistance. Others adsorb and steadily lower the resistance to 10^3 ohm cm.² or less, probably by penetration and/or formation of channels.

One, as yet unidentified, heat-stable molecule obtained from bacterially de-sugared egg whites which can be precipitated by ammonium sulphate (50–100 per cent sat.), denatured by chloroform and has an estimated mol. wt. of 10^4–10^6 adsorbs, lowers the resistance and, in addition, regularly induces the following gating reaction to d.c. electrical stimulation. At a sharp and generally unidirectional threshold voltage, which can be varied by [Ca^{++}] between 15 and 50 mV., the resistance shifts 5-fold to a new steady value after a latency which changes inversely with applied potential (Fig. 1). Recovery is prompt, the phenomenon repeatable and indistinguishable in detail from the behaviour of the excitable alga, *Valonia*, in the 'variable resistance' state[5] and is similar to the resistance increase of the skin 'action potential'[6] and to the frog nerve 'action potential' in isotonic potassium chloride[7]. The above resistance-voltage kinetics, including the threshold, can be predicted quantitatively from the general theory of reaction-rates under the assumptions that the adsorbed molecules form a finite number of two state resistive channels (that is, gates) and that the number in each state is determined by the Δ F. between the two states and the applied potential.

Horizontal BLMs on glass or teflon

Fertig, et al. 2001. *Phys Rev E* **64**, 040901; Mayer, et al. 2003. *Biophys. J.* **85**, 2684





Membranes can also be painted on holes in a horizontal film.

Modern versions of this technique are employed in some commercially available electrophysiology instruments (e.g., Nanion Orbit 16, Nanion Orbit Mini).

RAPID COMMUNICATIONS

PHYSICAL REVIEW E, VOLUME 64, 040901(R)

Microstructured glass chip for ion-channel electrophysiology

N. Fertig,[1,2] Ch. Meyer,[1,2] R. H. Blick,[1] Ch. Trautmann,[3] and J. C. Behrends[1,2]
[1]*Center for NanoScience and Sektion Physik, Ludwig-Maximilians-Universität, Geschwister-Scholl-Platz 1, 80539 München, Germany*
[2]*Physiologisches Institut, Ludwig-Maximilians-Universität, Pettenkoferstraße 12, 80336 Munich, Germany*
[3]*Gesellschaft für Schwerionenforschung, Planckstraße 1, 64291 Darmstadt, Germany*
(Received 18 May 2001; revised manuscript received 25 June 2001; published 21 September 2001)

We present a technique by which it is possible to produce a planar sensor for ion channel electrophysiology from glass substrates. Apertures with diameters in the low micrometer to submicrometer range are achieved by irradiation of a glass chip with a single heavy ion and subsequent wet track etching. The function of the device is demonstrated by recordings of single channel currents mediated by the model ion channel gramicidin A in lipid bilayers spanning the micromachined aperture.

DOI: 10.1103/PhysRevE.64.040901 PACS number(s): 87.80.Jg, 87.80.Mj, 87.68.+z

Ion channels play key roles in functions and dysfunctions of all cells. The most direct and accurate methods for studying ion channel behavior record the transmembrane current that results when channels open to allow ions to flow, while keeping the transmembrane voltage constant. The most precise of these voltage-clamp techniques is patch clamping [1,2], where a tight, high resistance seal is formed between the tip of an electrolyte-filled glass pipette and the cell membrane. This high resistance increases the resolution of recording so that currents mediated by few or even single open channels can be directly observed [3].

Recently, attempts are being made to replace the patch clamp pipette by planar chip-based sensors. Such an arrangement could facilitate automation and parallelization of ion channel recording when arrays of multiple sensors on a single chip are used to record from multiple membranes simultaneously. Moreover, a miniaturization of the sensor will reduce its electrical capacitance and, therefore, further increase resolution. Last, a planar geometry of the set up favors simultaneous use of optical or other techniques to study ion channels.

In order to take advantage of standard microstructuring techniques, silicon has been used as a substrate for such a device. However, this approach forgoes the superior electrical insulation provided by glass. The minimal requirement for an electrophysiological ion channel sensor is a small (micron-sized) aperture in an insulating material that separates two electrolyte-containing compartments. Drawing out a glass pipette to provide a micron-sized orifice is an elegant way of producing an aperture of small dimensions at the tip of a device that can be handled. However, this also produces a relatively long pathway through which the current must flow to the opening, leading to a considerable series resistance and capacitance of the device. It is also obvious that the fabrication procedure and the resulting geometry is unfavorable for producing arrays of such apertures.

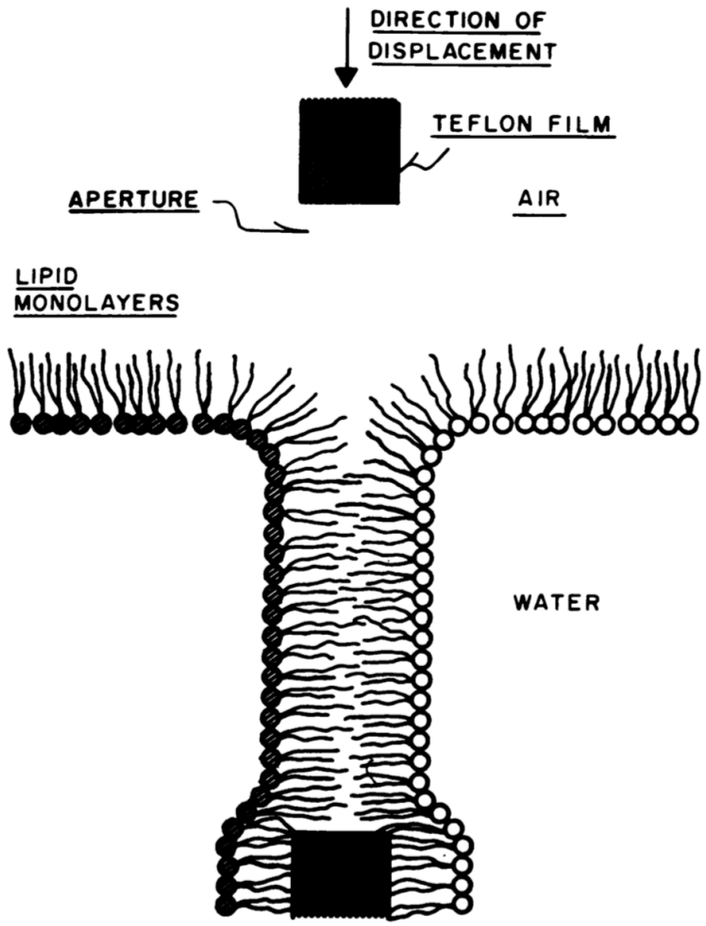
Even before the advent of the patch clamp technique, single ion channels were studied using planar lipid bilayers [4]. The apertures for bilayers are commonly produced in a thin teflon sheet by mechanical methods such as drilling. This perforated film is mounted as a diaphragm separating two solution-containing compartments. These apertures typically have diameters ranging from a few millimeters down to about 150 μm. The bilayer is composed either of solvent containing lipids [5] or of two solvent-free lipid monolayers [6]. Due to the large area of the resulting lipid membranes, their capacitance is rather high. The membrane capacitance in conjunction with the input voltage noise of the field effect transistor (FET) of the amplifier headstage plus the thermal voltage noise of the access resistance to the bilayer is mainly responsible for the noise level in the experiment [7]. In addition, the capacitance across the sheet containing the bilayer in a series with the input voltage noise of the FET must also be considered. Low-pass filtering is applied to achieve a suitable signal-to-noise ratio that limits the time resolution of an experiment. Consequently, reducing the size of the apertures and optimizing the geometry of the whole arrangement is most important for lowering the background noise. The development of the shaved aperture technique [8] enables the fabrication of apertures with sizes down to about 25 μm and improves the performance considerably. Up to now the best approach for low noise recordings from lipid bilayers is the so-called tip-dip technique [9,10], in which a bilayer is spread across the tip of a low resistance pipette, with tip diameter about 5–10 μm.

For achieving not only very fine apertures, but also to enable array fabrication in a parallel manner, advanced processing techniques from semiconductor technology can be applied. The standard material for the production of integrated circuits is silicon. Hence, in pilot studies by Fertig *et al*. [11], a silicon chip covered with silicon-nitride insulation layers was microstructured using conventional processing techniques. In this way, small orifices were realized. Initial conductance measurements on artificial bilayers formed by vesicle spreading were performed using a very similar approach by Schmidt *et al*. [12].

However, the glass materials used for patch pipettes are superior to semiconductor materials, due to their lower charge carrier density and the lower dielectric constant $\epsilon_Q = 4$ as compared to 10 for silicon, leading to much lower total capacitances. The main problem with glass and quartz so far is the difficulty in micromachining wafer materials. We describe below, the fabrication of submicron apertures using the ion-track etching technique for on-chip single-channel recording in detail. Such an ''on-chip pore'' in glass, fulfills in particular, the desired low noise requirements. Fi-

BLM "Solvent-Free" Method

Montal & Mueller. 1972. *PNAS* **69**, 3561



1. Add electrolyte to both chambers at a level well below the hole in a vertically-oriented Teflon film.
2. The hole is pretreated with an alkane (e.g., hexadecane or squalene) in pentane.
3. Lipid in pentane is spread at the air-water interface of each chamber. The pentane is allowed to evaporate (≈ 5 min).
4. The electrolyte level on each side is raised sequentially to just above the hole, and a BLM spontaneously forms in a thinning process like painted membranes (Niles, Levis, & Cohen. 1988. *Biophys. J.* **53**, 327)

Proc. Nat. Acad. Sci. USA
Vol. 69, No. 12, pp. 3561–3566, December 1972

Formation of Bimolecular Membranes from Lipid Monolayers and a Study of Their Electrical Properties

(membrane structure/membrane reconstitution/asymmetric membranes/lipid bilayers)

M. MONTAL* AND P. MUELLER†‡

* Departamento de Bioquimica, Centro de Investigacion y de Estudios Avanzados del I.P.N., Mexico 14, D.F. Mexico; and † Department of Basic Research, Eastern Pennsylvania Psychiatric Institute, Philadelphia, Pa. 19129

Communicated by Britton Chance, September 25, 1972

ABSTRACT **Bimolecular membranes are formed from two lipid monolayers at an air–water interface by the apposition of their hydrocarbon chains when an aperture in a Teflon partition separating two aqueous phases is lowered through the interface. Formation of the membrane is monitored by an increase of the electrical capacity, as measured with a voltage clamp. Electrical resistance of the unmodified membrane is analogous to that of conventional planar bilayers (black lipid membranes) prepared in the presence of a hydrocarbon solvent, i.e., 10^6–10^8 ohm cm^2; the resistance can be lowered to values of 10^3 ohm cm^2 by gramicidin, an antibiotic that modifies the conductance only when the membranes are of bimolecular thickness. In contrast to the resistance, there is a significant difference between the capacity of bilayers made from monolayers and that of hydrocarbon-containing bilayers made by phase transition; the average values are 0.9 and 0.45 μF cm^{-2}, respectively. The value of 0.9 μF cm^{-2} approximates that of biological membranes. Assuming a dielectric constant of 2.1 for the hydrocarbon region, the dielectric thickness, as calculated from a capacity of 0.9 μF cm^{-2}, is 22 Å. This value is 6–10 Å smaller than the actual thickness of the hydrocarbon region of bilayers and cell membranes, as determined by x-ray diffraction. The difference may be due to a limited penetration of water into the hydrocarbon region near the ester groups that would lower the electrical resistance of this region and reduce the dielectric thickness. Asymmetric membranes have been formed by adjoining two lipid monolayers of different chemical composition.**

Lipid bilayers, which are thought to be the basic structural element of cell membranes, account for many of their properties. They can be assembled from lipids either as small vesicles (1) or as single planar structures that separate two aqueous phases (2). Both models complement each other, and each has its own advantages and shortcomings. The spherical bilayers allow flux measurements with relative ease, and the absence of hydrocarbon solvent may be a factor aiding the incorporation of membrane proteins for functional reconstitutions (3–5). However, their inner compartment is small and inaccessible to chemical manipulation and electrical measurements. In planar bilayers, both compartments are easily accessible, but their mode of formation and the presence of hydrocarbon solvent may be responsible for reported failures to incorporate large membrane proteins. In addition, their electrical capacity is considerably lower than that of cell membranes, implying a different structure or thickness of the dielectric region.

For these reasons the formation of planar bilayers without the aid of a hydrocarbon solvent would be desirable. We report here the formation of planar bilayers separating two aqueous phases, in the absence of hydrocarbon solvent, by the hydrophobic apposition of two lipid monolayers at an air–water interface, by a modification of the method used by Takagi, Azuma, and Kishimoto (6) to form "rhodopsin membranes." It will be shown that the electrical capacity of these bilayers exactly matches that of biological membranes, and that the system allows the formation of asymmetric membranes; eventually, this technique may aid in the incorporation of membrane proteins into the lipid bilayer.

‡ To whom reprint requests should be addressed.

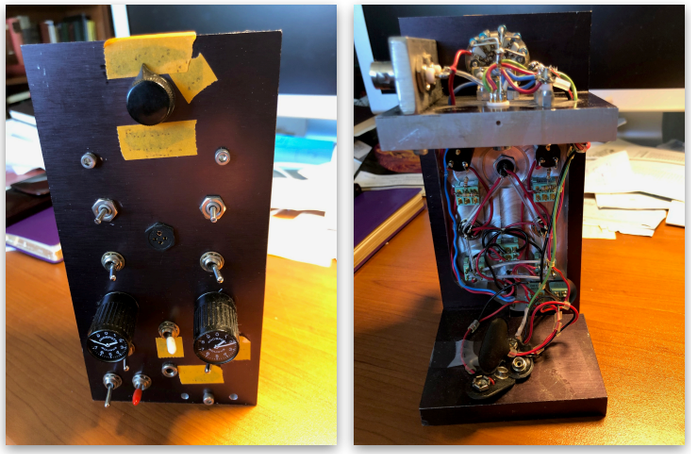
MATERIALS AND METHODS

The following chemicals were used: glyceroldioleate (Armak Chemicals Division, Philadelphia, Pa. and Applied Science Laboratories, Inc., State College, Pa.), oleyoyl acid phosphate (Hooker Chemical Co., Niagara Falls, N.Y.), egg lecithin, bovine cardiolipin, and plant phosphatidylinositol (Applied Science Laboratories, Inc.), gramicidin and cholesterol (Sigma Chemical Co., St Louis, Mo). Analytical grade reagents were used throughout. White-matter lipid from brain was extracted according to Mueller *et al.* (7) or by the method of Leuzinger and Schneider (8).

The membranes were formed initially with a modified version of the apparatus described by Takagi (9) (see Fig. 1*a*). It consists essentially of two monolayer troughs (18 × 12 cm) separated by a vertically movable septum containing in its center a hole of 1.0 cm (diameter) covered by a thin Teflon (tetrafluoroethylene) film (25 μm thick), obtained from Yellow Springs Instruments, Yellow Springs, Ohio (Membrane Kit no. 5937); this film contains a small aperture (see Fig. 1*a*). The septum is sealed with silicone grease to the walls of the trough and insulates the two water compartments electrically. It can be moved by a motor at a preset speed downwards, so that the aperture moves from above to below the water surface. The troughs and septum were made from Teflon. The aperture in the thin Teflon film was formed either by an electrically heated platinum wire, which was ground to a sharp point, or by a punch made from a tuberculin-syringe needle by beveling its wall. In a simplified version of this method, the thin Teflon film with the aperture was clamped between two halves of a trough and kept stationary. The membrane was formed by filling the two compartments with water or saline to below the aperture and, after spreading a lipid monolayer on each side, raising first one, then the other water level slowly above the aperture by gravity flow.

It is important that the film containing the aperture have a hydrophobic surface; this favors contact with the lipid hydrocarbon chains. Of several materials tested, only Teflon and

Electrophysiology Amplifier Examples



Home-made voltage clamp

Dagan 3900

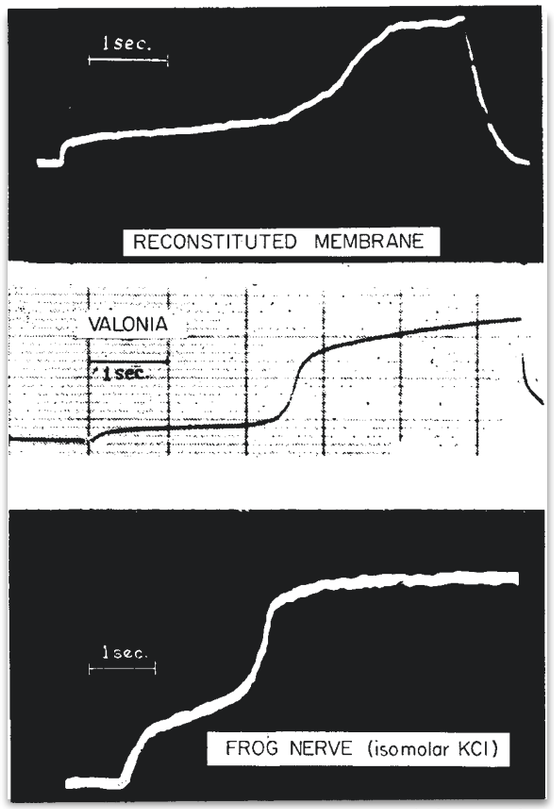
AxoPatch 200B

Heka epc 10 plus

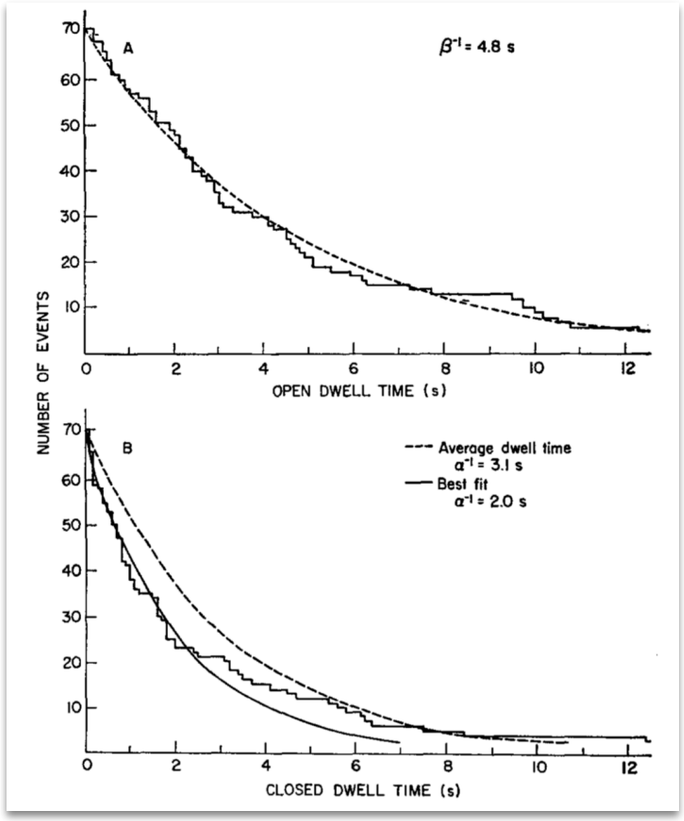
Nanion Orbit 16

Elements amplifiers

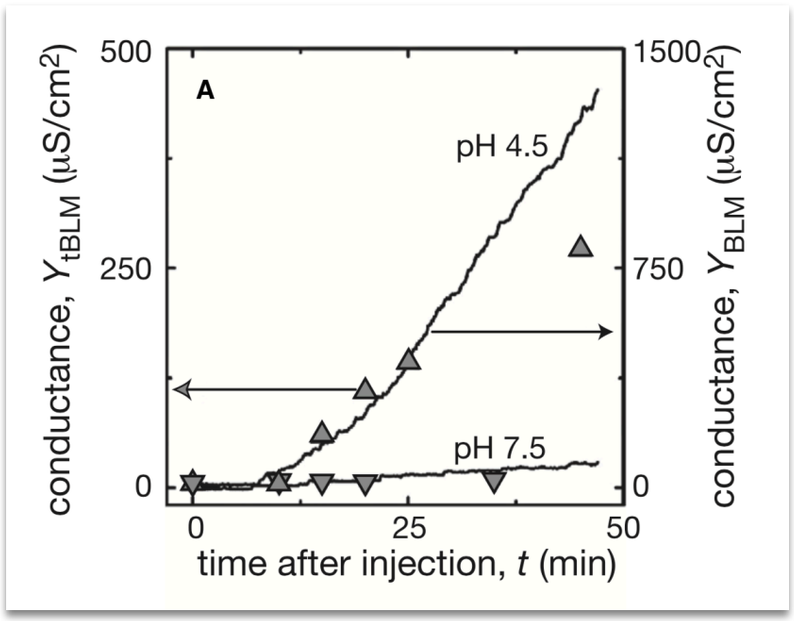
Typical Uses of BLM Technique



Reconstitution of cell membrane structure in vitro & its transformation into an excitable system
Mueller, Rudin, Tien, & Westcott. 1962. *Science* **194**, 979

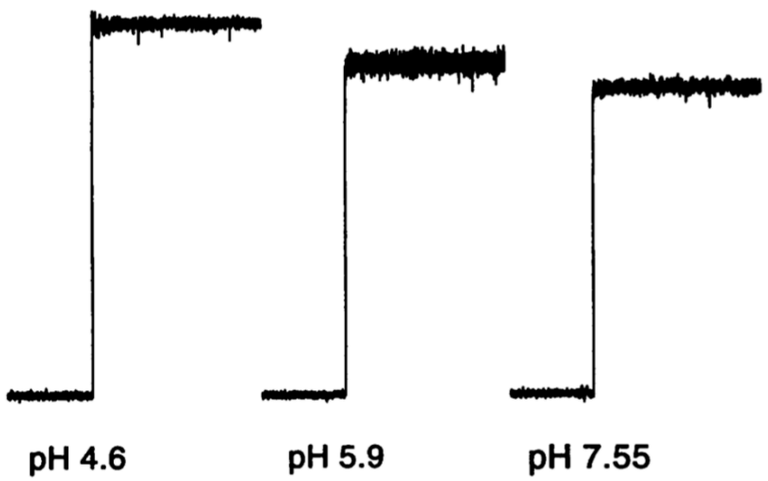


Ion channel opening & closing kinetics of "excitability inducing material, EIM"
Ehrenstein, Lecar & Nossal. 1974. *J Gen Physiol* **63**, 707

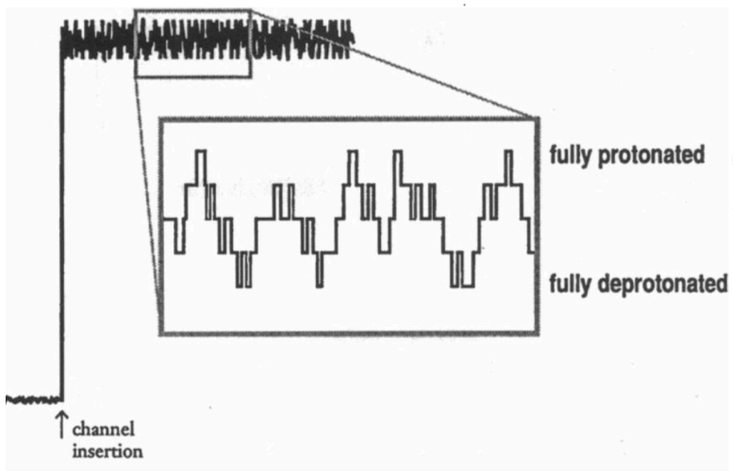


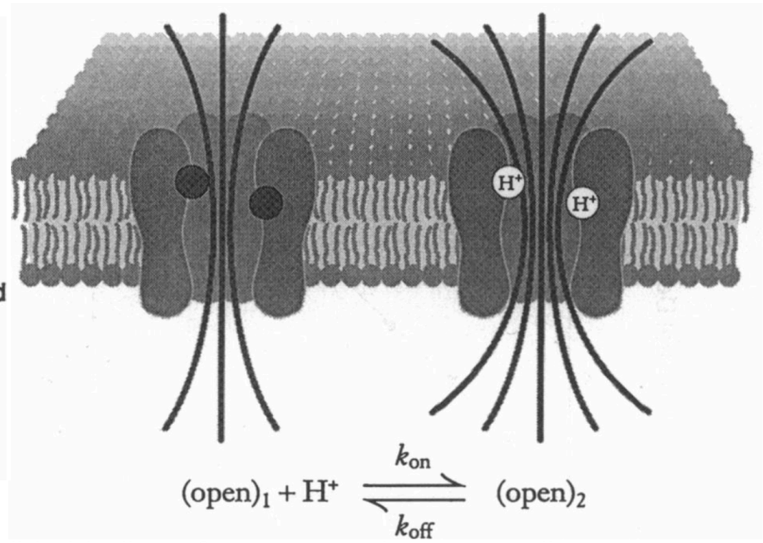
Kinetics of ion channel formation
McGillivray, et al. 2009. *Biophys J* **96**, 1547

Nanopore-based Single Molecule Detection

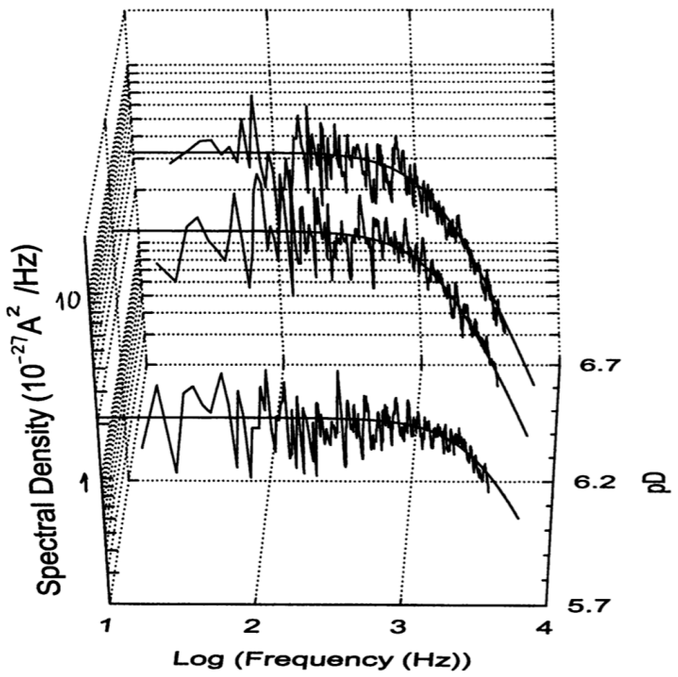


Ion current & current noise pH dependent



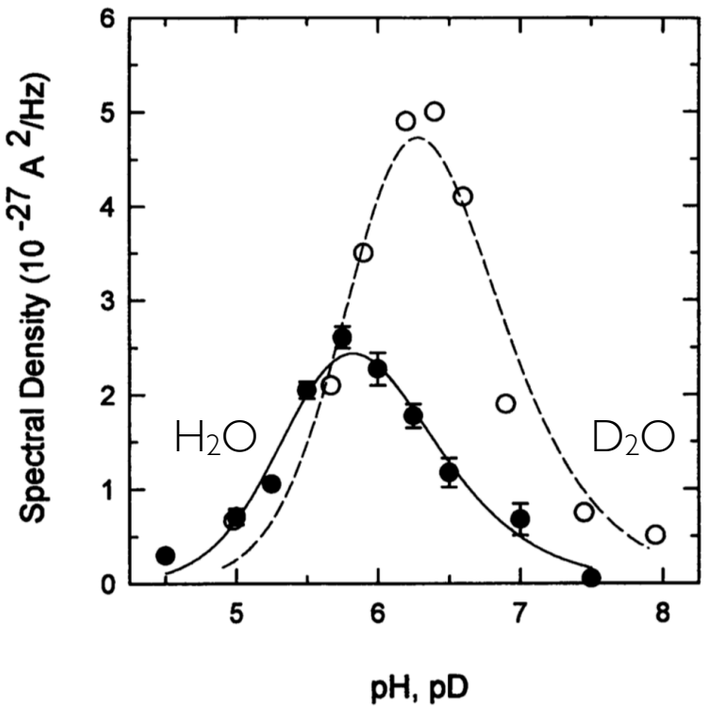


Current fluctuations caused by reversible binding of protons to the pore wall



Fast Fourier transform: ion current spectral density

Lorentzian lineshape: *simple reversible reaction*



Peak ~ pK_a

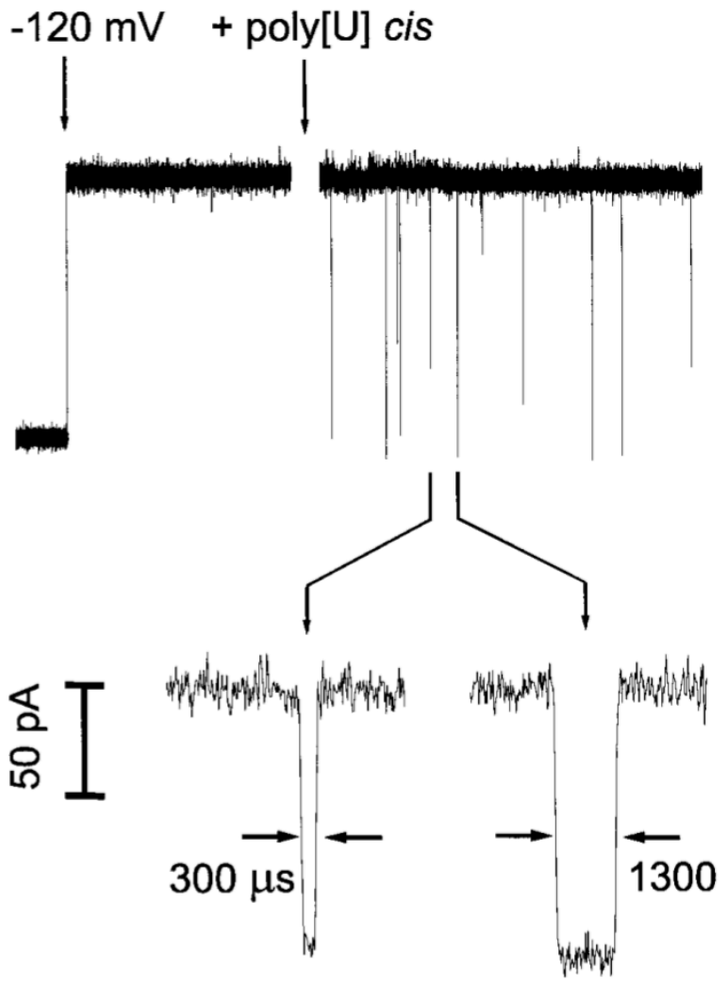
$K_a = k_{on}/k_{off}$

Low frequency spectral density quantitates pH dependent current noise

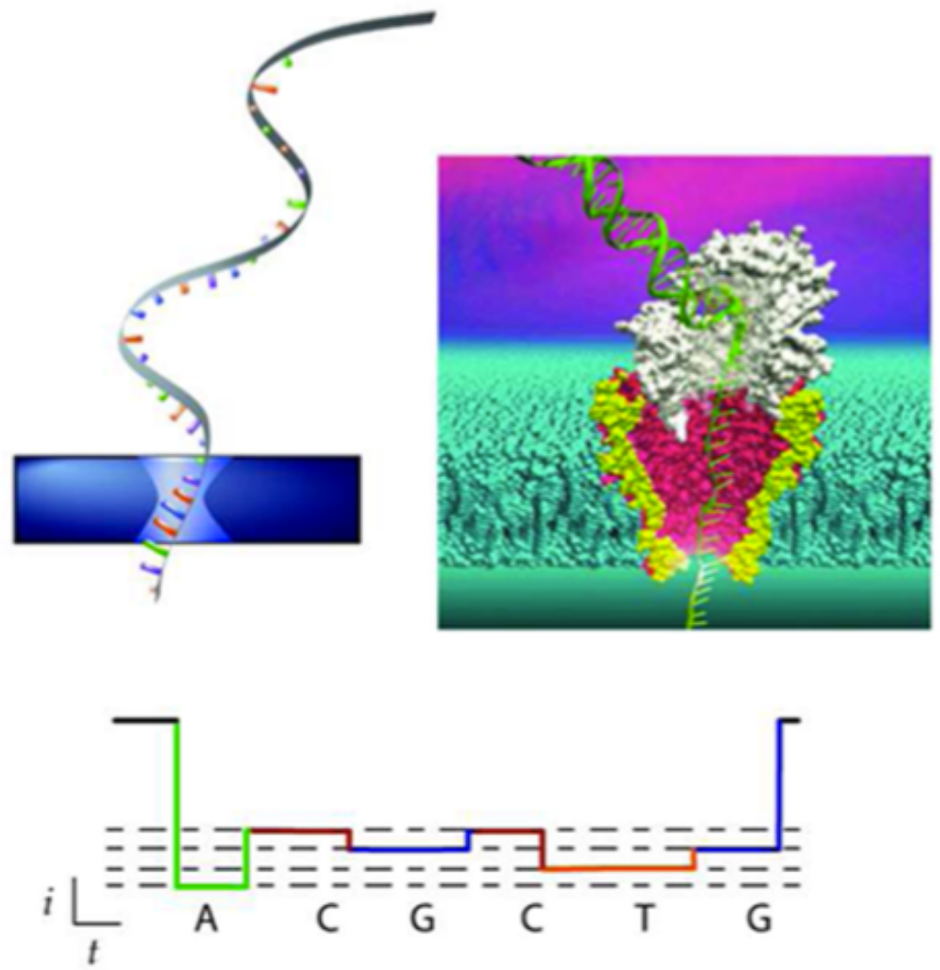
Bezrukov & Kasianowicz. 1993. *Phys Rev Lett* **70**, 2352; Kasianowicz & Bezrukov. 1995. *Biophys J* **69**, 94

Other Uses for Ion Channels

Nanopore-based RNA & DNA Oligonucleotide Detection

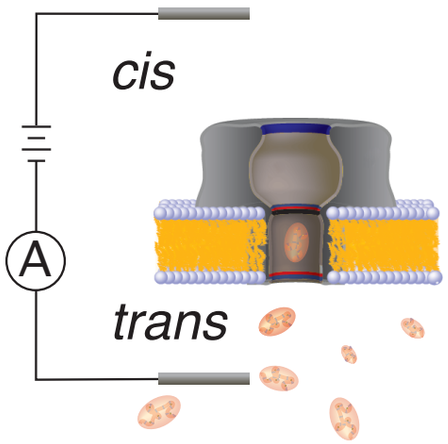


Individual polymers of single-stranded RNA cause well-defined ion current blockades.
Kasianowicz, et al. 1996. *PNAS* **93**, 13370

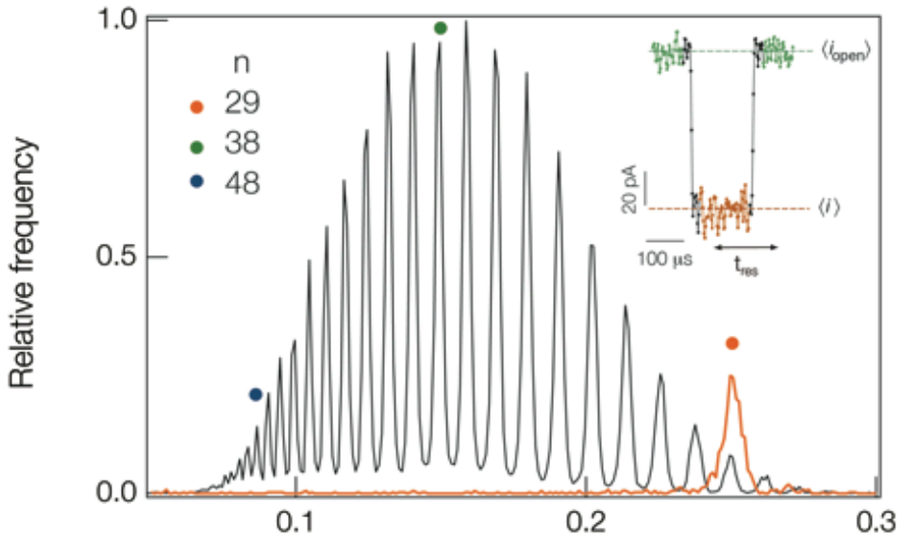


Provided part of the basis for nanopore-based DNA sequencing.
Further developed by Jens Gundlach's lab,
Oxford Nanopore Technologies, et al.

Nanopore-based Polymer Size Discrimination



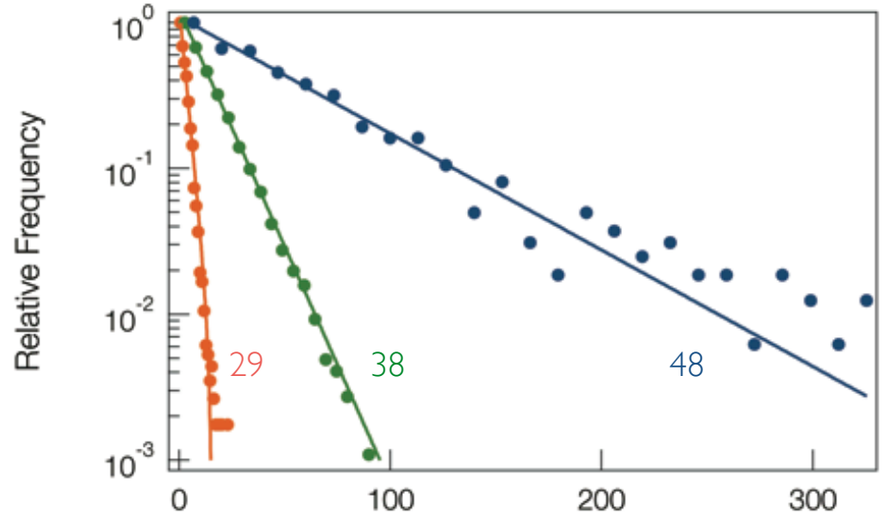
Poly(ethylene glycol) molecules transport into & out of the nanopore



Relative blockade depth $\langle i \rangle / \langle i_{open} \rangle$

Distribution of ion channel current blockades
polydisperse (black) or monodisperse (orange) PEG
Polymer free = 1, fully blocked = 0

Larger polymers reduce the current more than do smaller ones



Residence time (ms)

Distribution of residence times for 3 different size PEGs (29-, 38- & 48-mers)
Lifetimes follow single exponential kinetics
Suggests polymer + pore reaction is reversible

Larger polymer residence time > smaller polymer residence time

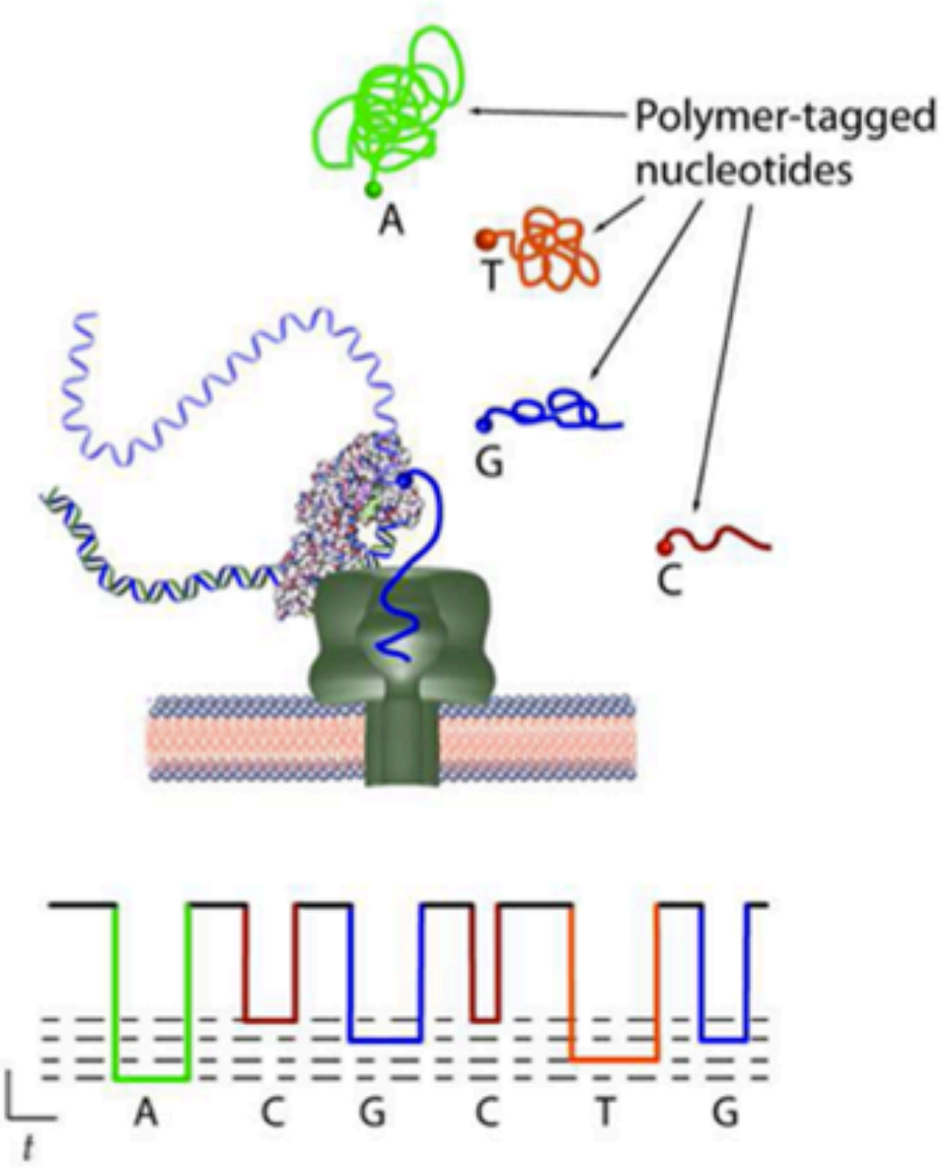
Robertson, Rodrigues, Stanford, Rubinson, Krasilnikov, & Kasianowicz. 2007. *PNAS* **104**, 8207

Nanopore-based DNA Sequencing by Synthesis

The ability to discriminate between different size polymers led to the development of DNA sequencing by synthesis with a single nanopore

collaboration with Jingyue Ju's group at Columbia University

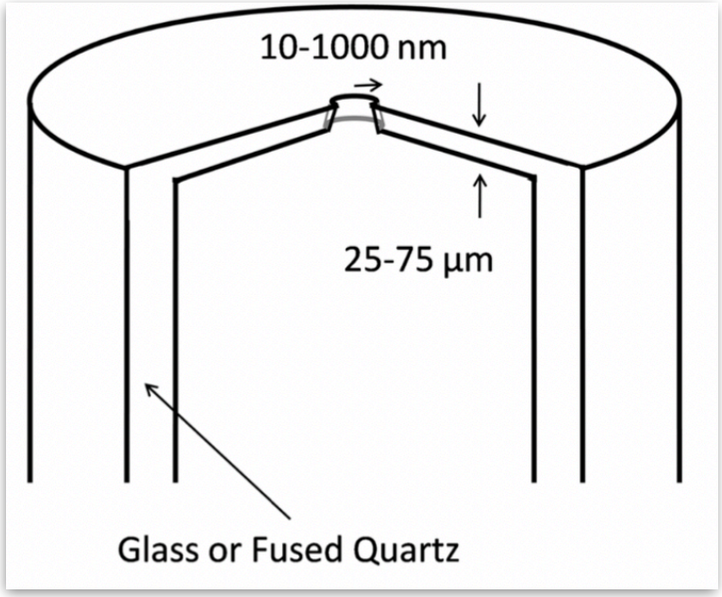
Now in use by Genia Technologies/Roche

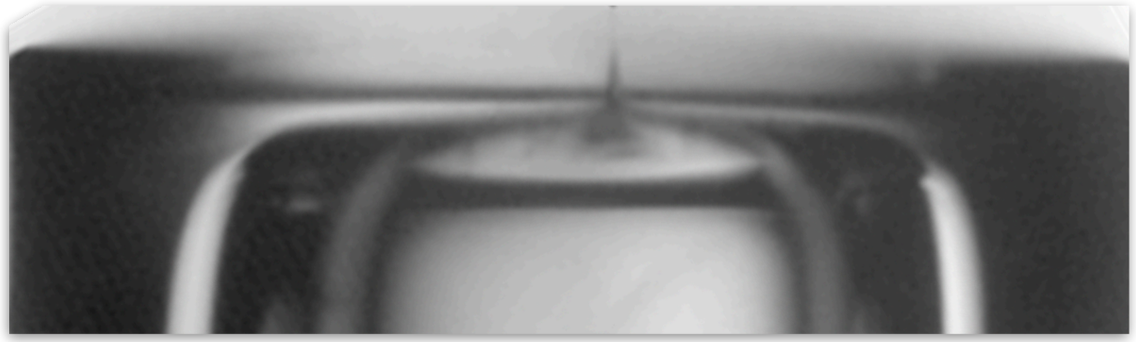


Kumar, et al. 2012. *Scientific Reports* **2**, 684; Fuller, et al. *PNAS* **113**, 5233

Electronic BioSciences Quartz Nanocapillary Membrane

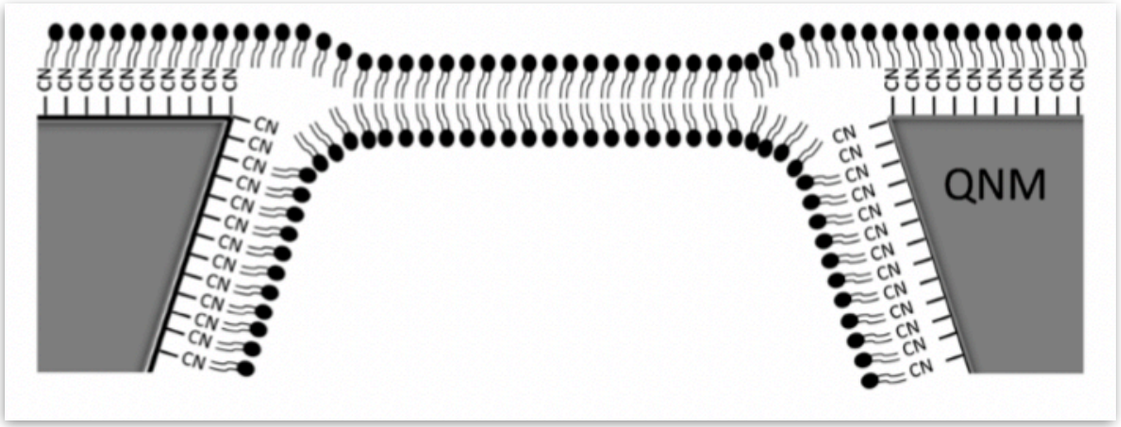
low capacitance membrane & support: high resolution & high bandwidth recordings



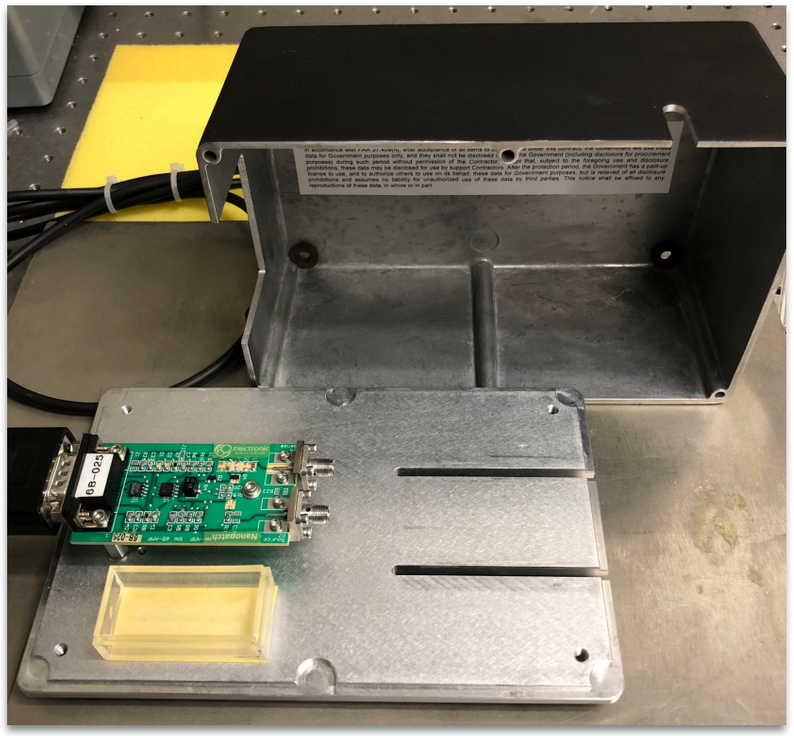


QNM micrograph

Schematic illustration of QNM

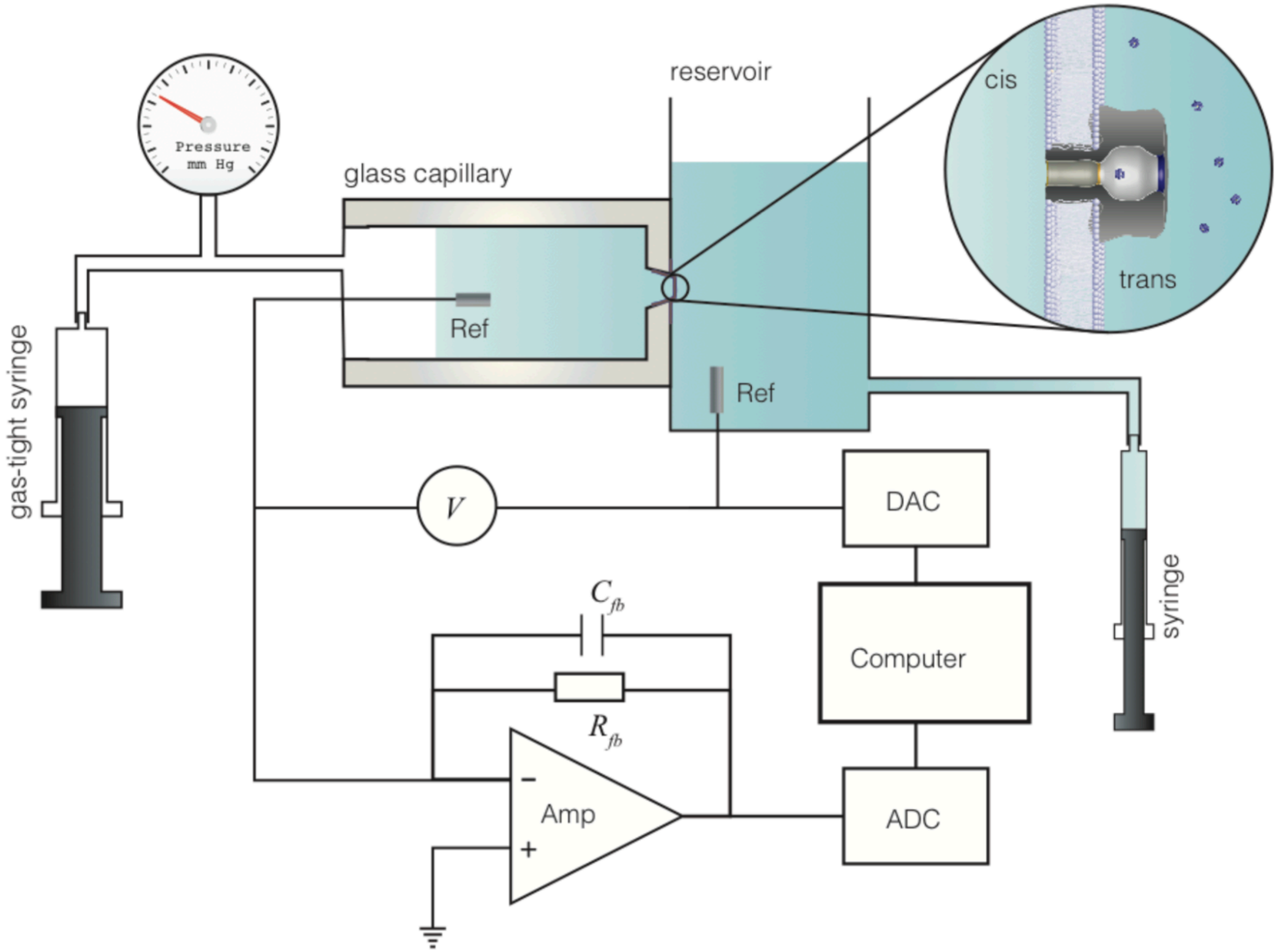


Cartoon illustration of a lipid bilayer formed on the QNM
(*not to scale*)



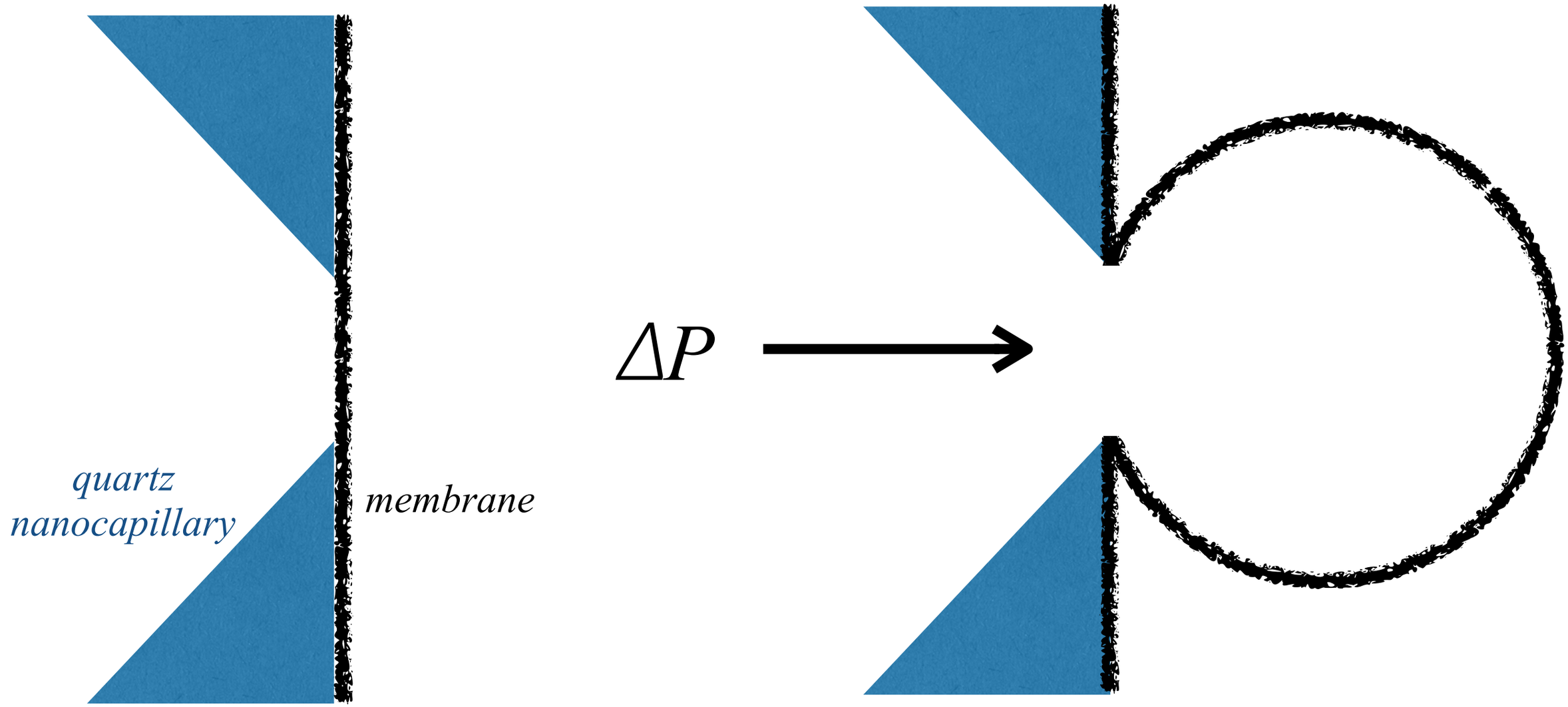
Typical EBS amplifier board & Faraday cage

Electronic BioSciences Rig Schematic



Ettedgui, et al. *JoVE*

Electronic BioSciences: Nanopore Reconstitution

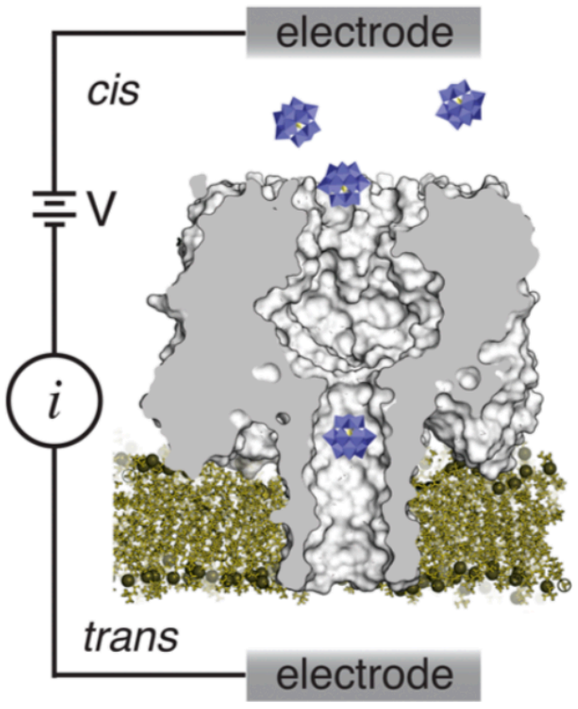


Apply transmembrane pressure: increase membrane surface area to increase probability of nanopore formation

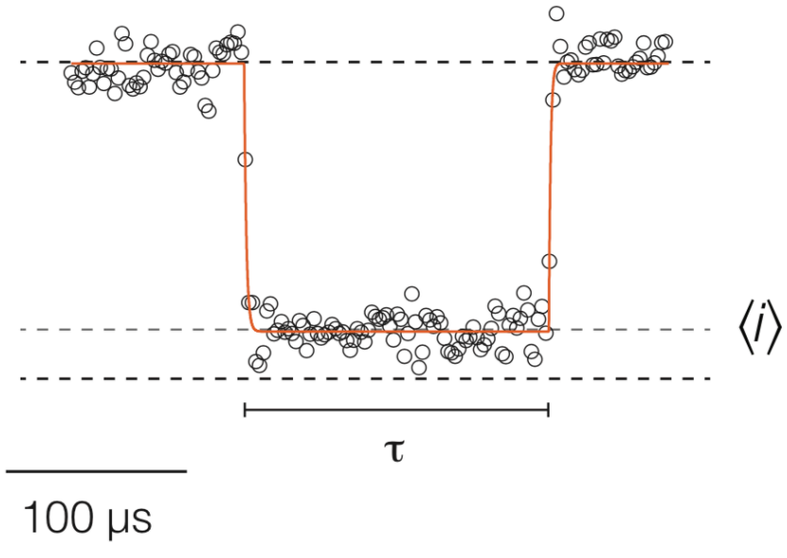
Electronic BioSciences Software

raw experimental results

Nanopore-based Detection of Individual Metal Nanoparticles

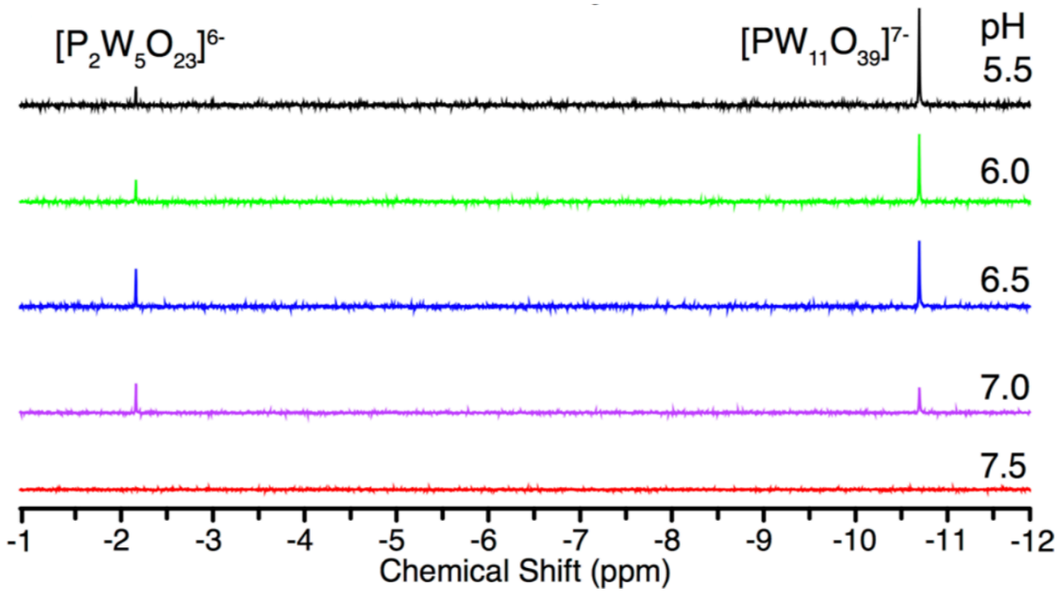


from Ettedgui, et al. 2016. *JACS* **138**, 7228



Ettedgui, et al. *JoVE*

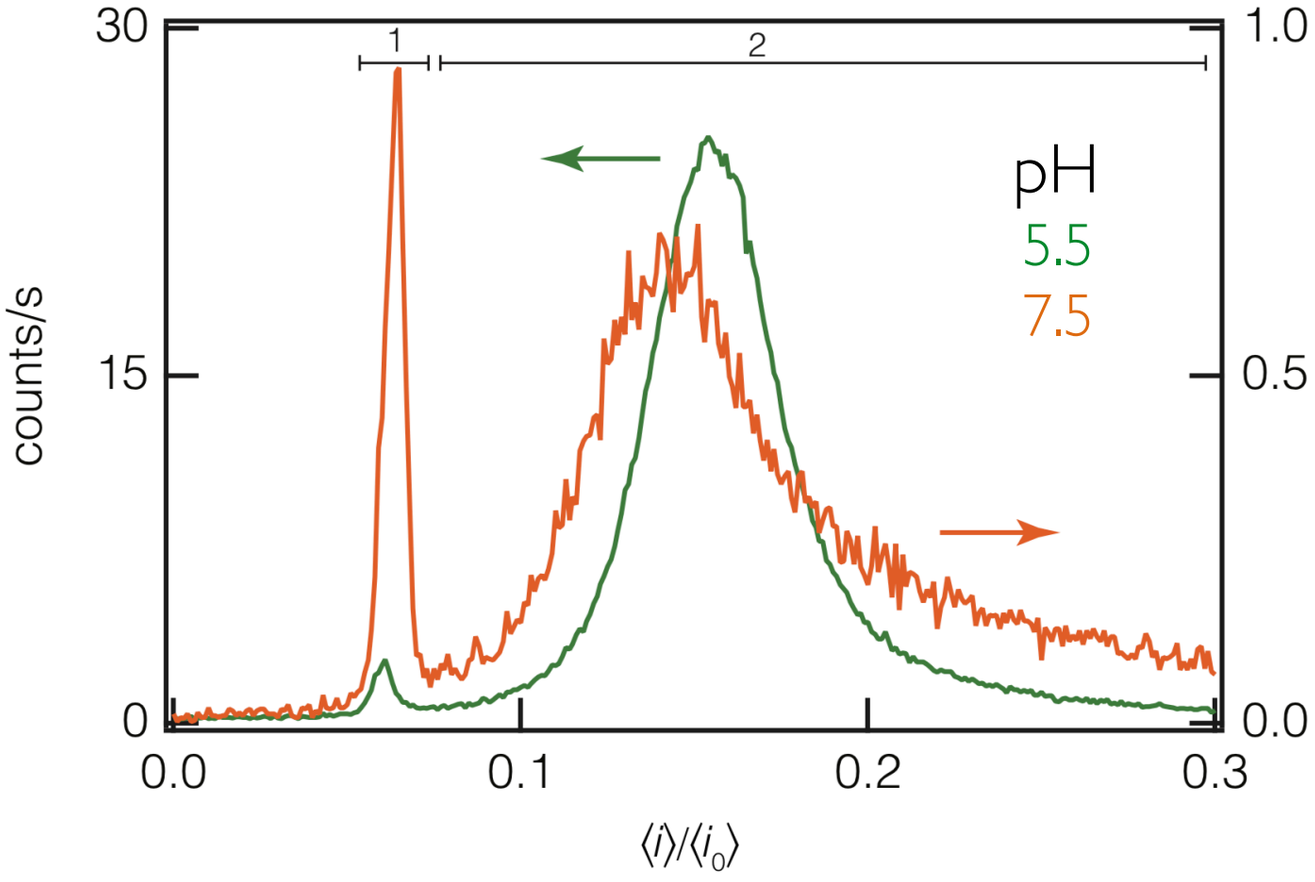
POM Species pH dependence



^{31}P NMR

Amounts of 2 principal species vary w/pH

from Ettedgui, et al. 2016. *JACS* **138**, 7228

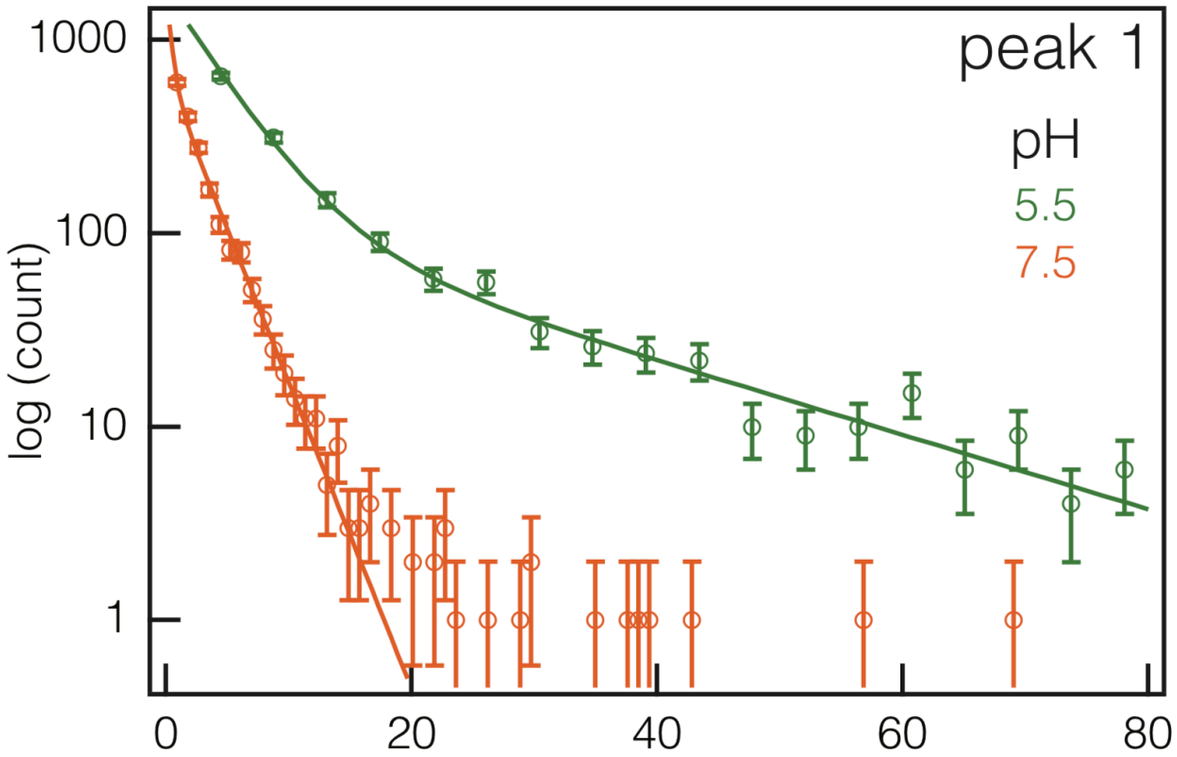


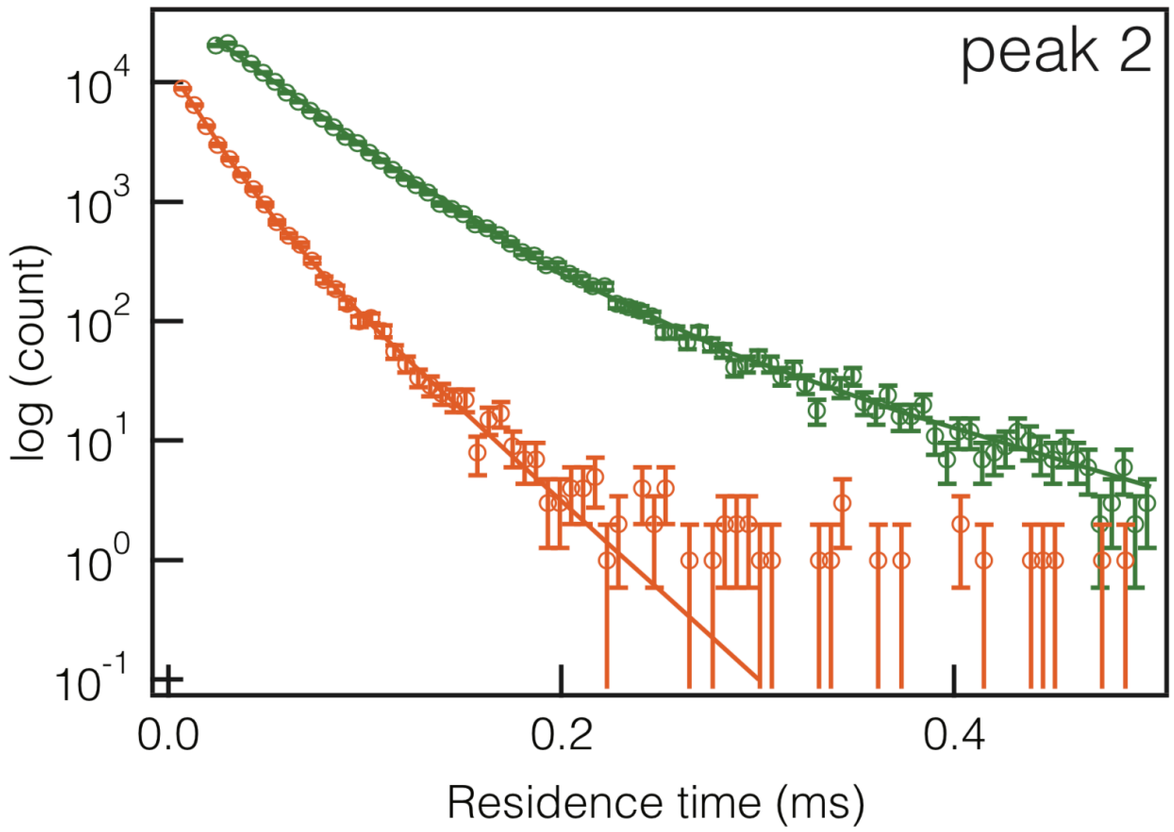
Ion current blockade depth distributions

Ettedgui, et al. *JoVE*

***Note**: nanopore detects species not seen by ^{31}P NMR at pH 7.5!*

Residence Time Distributions





$$\text{POM + pore} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{POM:pore}$$

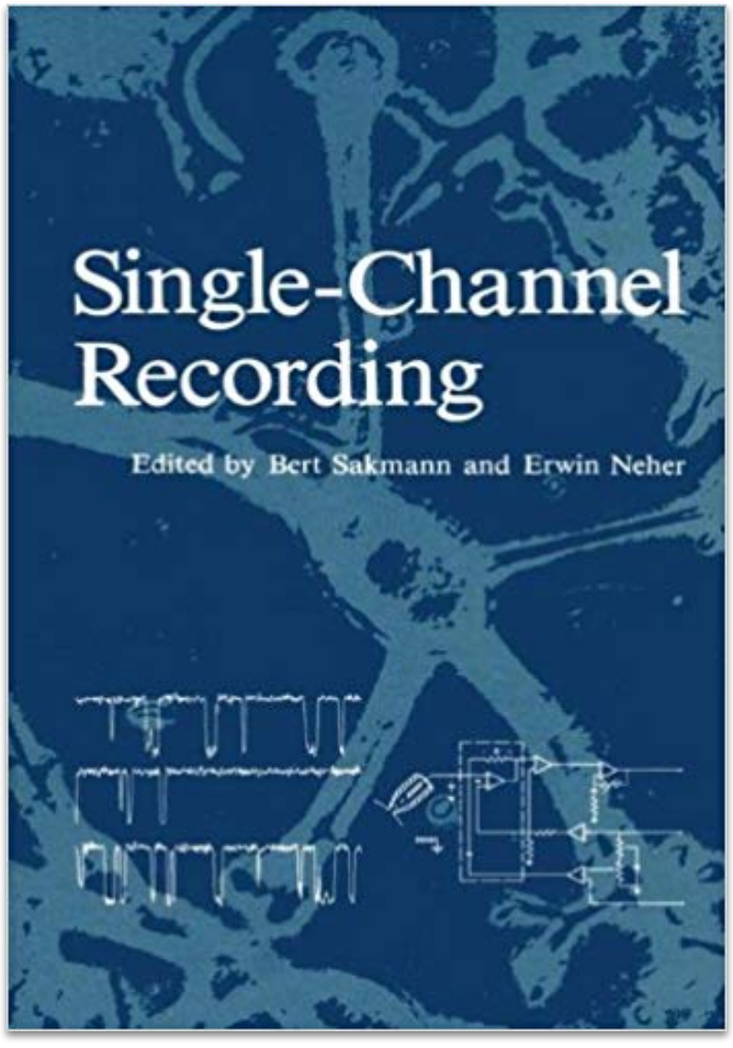
Simplest kinetic scheme: single decaying exponential

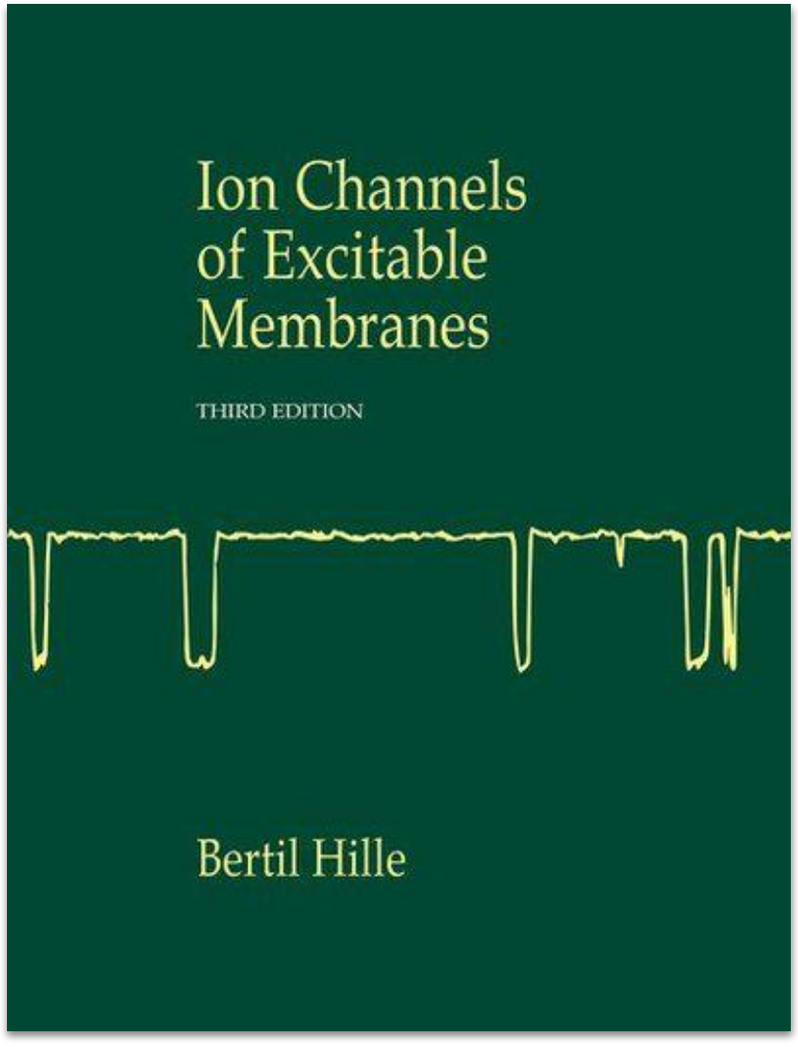
Multiple exponentials suggests multiple subspecies within each of the two peaks at both pH values

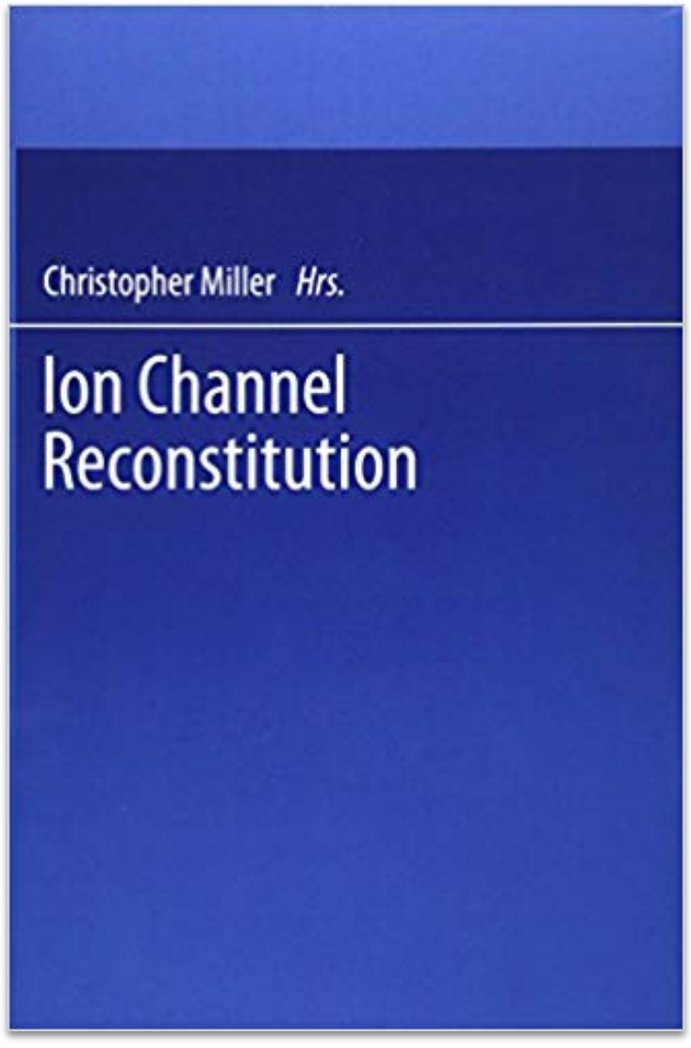
Ettedgui, et al. *JoVE*

More Information on Electrophysiology

books







Nanopore-based single molecule analysis

some reviews

Nanoscopic Porous Sensors

John J. Kasianowicz,[1] Joseph W.F. Robertson,[1] Elaine R. Chan,[1] Joseph E. Reiner,[1] and Vincent M. Stanford[2]

[1]National Institute of Standards and Technology, Semiconductor Electronics Division, Electronics and Electrical Engineering Laboratory, Gaithersburg, Maryland 20899-8120; email: john.kasianowicz@nist.gov

[2]National Institute of Standards and Technology, Information Access Division, Information Technology Laboratory, Gaithersburg, Maryland 20899-8940

Key Words

analyte detection, Coulter counter, DNA sequencing, ion channel, nanopore-based sensor, resistive-pulse detection

Abstract

There are thousands of different nanometer-scale pores in biology, many of which act as sensors for specific chemical agents. Recent work suggests that protein and solid-state nanopores have many potential uses in a wide variety of analytical applications. In this review we survey this field of research and discuss the prospects for advances that could be made in the near future.

Kasianowicz, et al. 2008. *Ann Rev Analyt Chem* **1**, 737

Nanopore analytics: sensing of single molecules

Stefan Howorka[a] and Zuzanna Siwy[b]

Received 17th February 2009
First published as an Advance Article on the web 15th June 2009
DOI: 10.1039/b813796j

In nanopore analytics, individual molecules pass through a single nanopore giving rise to detectable temporary blockades in ionic pore current. Reflecting its simplicity, nanopore analytics has gained popularity and can be conducted with natural protein as well as man-made polymeric and inorganic pores. The spectrum of detectable analytes ranges from nucleic acids, peptides, proteins, and biomolecular complexes to organic polymers and small molecules. Apart from being an analytical tool, nanopores have developed into a general platform technology to investigate the biophysics, physicochemistry, and chemistry of individual molecules (*critical review*, 310 references).

1. Introduction

The sensing of individual molecules has become an important research component within modern biochemistry, biophysics, and chemistry.[1] Detecting individual molecules can also lead to the development of next-generation bioanalytical and diagnostic tools.[2,3] Among the group of single-molecule techniques, sensing with nanopores is likely the youngest member. Despite its recent inception, the field of nanopore research has increased exponentially within the past 10 years and has become widely accepted.[4] One of the reasons for the popularity is the simplicity of the approach. In nanopore sensing, individual molecules pass through a nanoscale pore leading to detectable changes in ionic pore current. The approach is label-free and does not require the analytes to be immobilised on a surface. Nanopore analytics has also become popular as it has the potential to sequence DNA strands at high speed and low cost.[2] While this aim has so far not been achieved, the range of analytes that can be detected with nanopores now spans small molecules, organic polymers, peptides, proteins, enzymes, and biomolecular complexes. Indeed, nanopore recording has developed from a simple sensing tool into a general platform technology which enables the examination of the physicochemistry, biophysics, and chemistry of individual molecules. The proliferation in scientific use has been accompanied by an increase in the variety of nanopores. While initial experiments were solely performed with natural protein pores, man-made nanopores in organic polymers or inorganic materials such as silicon nitride, silicon dioxide, silicon, or silicate are now used on a routine basis. The general use of engineered biological and solid-state

[a] *Department of Chemistry, Institute of Structural Molecular Biology, University College London, London, UK WC1H 0AJ. E-mail: s.howorka@ucl.ac.uk*
[b] *School of Physical Sciences, University of California at Irvine, 210G Rowland Hall, Mail Code: 4575, Irvine, CA 92697, USA. E-mail: zsiwy@uci.edu*

Stefan Howorka

Stefan Howorka obtained his PhD degree from the University of Vienna and worked at Texas A&M University as a postdoctoral fellow. After a stint at an Austrian biotech incubator, he joined University College London, Department of Chemistry as a Lecturer in 2005. His current research interests include the rational design of biomaterials by subjecting porous proteins to genetic engineering and chemical modification, the use of designed biomaterials in single-molecule detection schemes, and nanopatterning and surface modification.

Zuzanna Siwy

Zuzanna S. Siwy received her PhD and habilitation in physical chemistry from the Silesian University of Technology in Gliwice, Poland. In 2000–2003 she was a Fellow of the Foundation for Polish Science, and the Alexander von Humboldt Foundation at the Institute for Heavy Ions Research (GSI) in Darmstadt, Germany. After conducting postdoctoral research in the group of Prof. C. R. Martin at the Department of Chemistry, University of Florida, Zuzanna Siwy joined the Department of Physics and Astronomy at the University of California, Irvine where she has been recently promoted to Associate Professor. Her current research interests focus on using synthetic nanopores as templates for biomimetic channels as well as ionic diodes and ionic transistors.

Howorka & Ziwy. 2009. *Chem Soc Rev* **38**, 2360

CHEMICAL REVIEWS

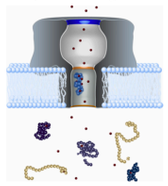
Review

pubs.acs.org/CR

Disease Detection and Management via Single Nanopore-Based Sensors

Joseph E. Reiner,*[,†] Arvind Balijepalli,[‡,§] Joseph W. F. Robertson,[‡] Jason Campbell,[‡] John Suehle,[‡] and John J. Kasianowicz[‡]

[†]Department of Physics, Virginia Commonwealth University, 701 W. Grace Street, Richmond, Virginia 23284, United States
[‡]Physical Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8120, United States
[§]Laboratory of Computational Biology, National Heart Lung and Blood Institute, Rockville, Maryland 20852, United States



CONTENTS

1. INTRODUCTION

1.1. Channels and Porins: Current and Possible Future Roles

As nanometer-scale portals in biological membranes, protein ionic channels act as gatekeepers, controlling the traffic of ions and macromolecules into and out of cells, organelles, and the nucleus. Because of their ubiquitous nature, proper channel function is critical to all aspects of life. One might suppose that the most obvious feature of these transmembrane proteins, a nanometer-scale hole in a ca. 4 nm thick phospholipid bilayer membrane,[1,2] renders channels as the simplest of biological machines. However, channels have evolved in rather sophisticated ways to control a wide range of biological function. We briefly discuss below some of the roles channels play in biology, as well as why they and mimics of them are emerging as effective biosensors for characterizing and quantifying many types of molecules. We then describe some examples of how such a novel measurement capability could prove useful for detecting disease states, assessing the efficacy of therapeutic agents, and managing the treatment of human disease.

Special Issue: 2012 Ion Channels and Disease

Received: September 13, 2012
Published: November 16, 2012

dx.doi.org/10.1021/cr300381m | *Chem. Rev.* 2012, 112, 6431–6451

Reiner, et al. 2012. *Chem Rev* **112**, 6431

ACS SENSORS

Review

Cite This: *ACS Sens.* 2018, 3, 251–263

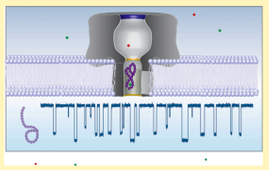
pubs.acs.org/acssensors

Determining the Physical Properties of Molecules with Nanometer-Scale Pores

Haiyan Wang,[†,‡] Jessica Ettedgui,[†,§] Jacob Forstater,[†,§] Joseph W. F. Robertson,[†] Joseph E. Reiner,[∥] Huisheng Zhang,[‡] Siping Chen,[#] and John J. Kasianowicz*[,†,⊥]

[†]National Institute of Standards and Technology Physical Measurement Laboratory, Gaithersburg, Maryland 20899, United States
[‡]Shenzhen Key Laboratory of Biomedical Engineering, School of Medicine, Shenzhen University, 3688 Nanhai Road, Shenzhen 508060, China
[§]Department of Chemical Engineering, Columbia University New York, New York 10027, United States
[∥]Department of Physics, Virginia Commonwealth University, Richmond, Virginia 23284, United States
[⊥]Department of Applied Physics Applied Mathematics, Columbia University New York, New York 10027, United States

ABSTRACT: Nanometer-scale pores have been developed for the detection, characterization, and quantification of a wide range of analytes (e.g., ions, polymers, proteins, anthrax toxins, neurotransmitters, and synthetic nanoparticles) and for DNA sequencing. We describe the key requirements that made this method possible and how the technique evolved. Finally, we show that, despite sound theoretical work, which advanced both the conceptual framework and quantitative capability of the method, there are still unresolved questions that need to be addressed to further improve the technique.



KEYWORDS: *nanopores, ion channels, sensors, analyte detection, DNA sequencing, microRNA, protein−DNA interactions, chemically modified nanopore, gold nanoclusters, metallonanoclusters*

The ability to detect and characterize single biological molecules provides insight into their physical properties and molecular mechanisms of action that cannot be gained from ensemble measurements. Conventional single molecule spectroscopy methods are based on the measurement of mechanical forces (AFM)[1,2] and light (fluorescence).[3,4] However, it was shown that individual molecules could also be probed by measuring their effect on the ionic current that otherwise flows freely through single ion channels.[5] The conceptual basis for this method was partially founded in the ability of receptor channels at neural synapses to discriminate between different neurotransmitters. These channels, and others that are the basis for nerve and muscle activity,[6−10] function by gating (i.e., switching) between discrete conducting states, open and close in response to a physical stimulus (e.g., the binding of a neurotransmitter) in a dose-dependent manner.

Experimental evidence suggested that sensing individual analytes based on how the target molecules influence the "gating" behavior of channels was possible.[11,12] For example, it was shown that hydronium and deuterium ions in aqueous solution could be discriminated based on the ionic current fluctuations they caused in the channel formed by *Staphylococcus aureus* alpha-hemolysin (αHL).[11,12] Those positive results notwithstanding, there were major hurdles to mimicking receptor channels as biosensors. For example, designing nanopores that bind specific analytes and convert the interaction into predictable channel gating events is a daunting task.

Another seemingly simple method for nanopore-based sensing of analytes was to measure the ionic current in response to the reversible transport of molecules into and out of the channel pore. This concept, while compelling, is naïve. First, any spontaneous gating that occurs in the absence of the target analyte would complicate the use of channels as sensors. This problem was overcome when it was shown that the voltage-dependent gating in the αHL channel could be essentially eliminated.[11−13] Second, many channels are so narrow that only water and small ions can enter them. However, Krasilnikov and colleagues demonstrated that the αHL channel was sufficiently wide that polymers of ethylene glycol with molecular mass ≤ 2000 g mol^{-1} could partition into the pore.[14,15] Third, assuming one-dimensional Brownian motion[16] of a polymer through the channel and a channel length ca. 4 nm (i.e., the width of a lipid bilayer membrane), the

Received: September 12, 2017
Accepted: January 8, 2018
Published: January 30, 2018

DOI: 10.1021/acssensors.7b00680
ACS Sens. 2018, 3, 251−263

Wang, et al. 2018. *ACS Sensors* **3**, 251