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# *MATHEMATICAL, PHYSICAL AND ENGINEERING SCIENCES DIVISION*

## MEMBRANE PORES

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## ABSTRACT

Every cell in the body is surrounded by a narrow membrane that consists of lipids and proteins. The lipids provide insulation from the external environment, the proteins play an opposite role: they sense the environment and transmit information to the interior of the cell. In many cases the signals are transmitted through narrow pores across the membrane: the propagation of impulses in nerves and the contraction of muscles are examples. Different types of protein make different kinds of pore: some for the passage of specific inorganic ions like K+, Na+ and $Ca^{2+}$, others for the passage of organic nutrients, like glucose and amino acids.

Membrane pores across the cell membrane are also induced by a variety of toxic agents. In this case the pores are non-specific and the outcome is detrimental to the life of the cell, since essential ions and molecules leak out. Agents as diverse as viruses, bacterial and animal toxins, low concentrations of detergents and other molecules cause such pores to be formed. We have found that these types of pore have certain properties in common. One is that pores are closed by divalent cations: the action of zinc in this regard may play a role in fighting off infections caused by some viruses and bacteria. Another property is that if a voltage difference is applied across the pore, current does not flow continuously, but oscillates between high and low conductance states. Such fluctuations of current are typical also of the endogenous ion channels for $K^+$, for $Na^+$ and for $Ca^{2+}$, mentioned above.

In order to better understand the nature of these effects, we have studied pores created across synthetic membranes made of organic polymers like polyethylene-terephthalate (PETP). Surprisingly, these too show fluctuations of current and inhibition by divalent cations. Such results provide new insights into the mechanisms underlying the flow of ions and molecules through biological membrane pores. They also open up the possibility of using synthetic membranes in various novel situations.

## INTRODUCTION

Every cell in the body is surrounded by a narrow membrane that consists of lipids and proteins. The lipids provide insulation from the external environment, the proteins play an opposite role: they sense the environment and transmit information to the interior of the cell. In many cases the signals are transmitted through narrow pores across the membrane: the propagation of impulses in nerves and the contraction of muscles are examples. Different types of protein make different kinds of pore: some for the passage of specific inorganic ions like $K^+$, $Na^+$ and $Ca^{2+}$, others for the passage of organic nutrients like glucose and amino acids. The proteins that are part of the membrane pore act catalytically, like enzymes: they speed up the diffusion of ions and molecules across the membrane.

### Endogenous ion channels

The leakage of cations from one side of a lipid-containing, high dielectric membrane to the other side generates a voltage difference across the membrane. Thirty years ago a methodology for measuring the current carried by ions passing through a single pore or channel was devised by two German scientists, Erwin Neher and Bert Sackmann (who were rewarded with the Nobel prize for their achievement). Surprisingly it turned out that current flow is not continuous, but fluctuates between high and low conductance states. The interpretation of this result is that channels appear to 'open' and 'close' insome way. Electron microscopy and X-ray crystallography of channel proteins supports this view.

### Induced membrane pores

It has been known for many years that disease resulting from a bacterial infection is often due not to the multiplication of bacteria inside host cells. On the contrary, this type of pathogenic mechanism, exemplified by the *Mycobacteria* – that give rise to leprosy and tuberculosis – is in the minority. The majority of microbial diseases are due to protein toxins released by bacteria: *Streptococci, Staphylococci. Salmonella* and many others produce a variety of protein toxins that give rise to food poisoning and blood poisoning, to respiratory and neurological diseases, and a host of other ailments. All disease is due to damage or death of cells within particular organs of the body. In the case of *Mycobacteria* (as well as of all viruses and protozoa such as the malarial parasite), the injury is caused by the presence of the invading organism within cells. In the case of *Streptococci* and other bacteria that multiply outside cells, the injury is caused by the toxic proteins they secrete.

How do such toxins disrupt the machinery of cells? The molecular details vary from toxin to toxin, but all have in common an interaction with the proteins or lipids that constitute the cell membrane. In some cases, it is the presence of a part of the toxic protein that enters cells and that then inflicts the damage by

inhibiting an important metabolic process, such as protein synthesis: diptheria toxin, cholera toxin, tetanus toxin and botulinum toxin are examples of this type of action. In other cases, the toxic proteins form pores across the cell membrane: the pores are sufficiently long-lasting for small molecules and ions to leak out of the affected cell: their loss is the cause of cellular injury. The pores resemble the endogenous ion channels mentioned earlier, in that they create an aqueous continuity between the inside and the outside of cells. They differ in two important aspects: toxin-induced pores are larger and are non-specific, allowing any ion or molecule below a certain size to leak through them. Whereas the diameter of an endogenous ion channel is in the order of angstroms, that of a microbially induced pore is typically 10 to 100 times larger.

Two other properties of induced membrane pores are relevant to this discussion. The first is that when a voltage difference is applied across a single membrane pore, the ensuing ion current is not continuous, but fluctuates between states of high and low conductance just as in an endogenous ion channel. This is surprising, given the large size of some of these pores: can subtle variations in protein structure really close a pore that is some 10 nm in diameter? We shall return to this question later. The second property is that in many cases the flow of ions and small molecules through a pore is inhibited by the presence of divalent cations. Pores created by low concentrations of detergent or by polycations show a similar effect. Protons, in other. words low pH, has a similarly protective effect and this is true of certain endogenous ion channels also. Zinc, which is a relatively non-toxic ion, is a particularly effective divalent cation and may play a role in ameliorating certain bacterial and viral infections as a result of this action. In order to investigate these properties further, we turned to study pores across synthetic membranes made of plastic.

**Synthetic pores**

If sheets of plastic made of synthetic polymers like polycarbonate or polyethylencterephthalate (PETP) are exposed to bombardment by a beam of heavy ions, tracks are formed across the membrane. The tracks can be expanded into pores by 'etching' in warm alkali, which breaks the ester bonds within the polymer Pores can be created in this way with diameters from 2-3 nm upwards, depending on the length of time that the membrane is exposed to etchant; in other words pores can be created across a PETP membrane that are similar in width to those of pores induced across a biological membrane. The length of PETP pores, however, is different. The thickness of PETP membranes is typically around 10 nm, whereas that of biological membranes is 1,000 less. When we studied the properties of PETP pores, we were surprised to find them similar to those of endogenous ion channels and toxin-induced pores. First, current fluctuates between high and low conductance states; second, current is inhibited by low pH and by divalent cations, third, current is selective, being carried predominantly by cations (a property that is particularly marked in endogenous ion channels. It seems that the width of

pores is more important than their length. If PETP is etched for longer times, creating wider pores, the distinctive properties of fluctuation of current, inhibition by protons and divalent cations, and ionic selectivity, disappear.

But there is a problem. Numerically, the conductance through a PETP pore is approximately the same as that through an endogenous ion channel: it is in pS. Yet a PETP pore is 1,000 longer, so its resistance should be 1,000 greater, and its conductance 1,000 less. We interpret the discrepancy as being due to the phenomenon of 'surface conductance', first described a century ago by a Polish scientist named Smoluchowski. Surface conductance is due to the fact that fixed charges along a surface attract ions of opposite charge, giving a greater than expected conductance. In the case of etcher PETP, the negative charges due to carboxylate groups attract cations. The result is that in a solution of KCl, for example, the concentration of K+ at the walls of the pore is hundreds of times greater than in bulk solution, and hence the conductance is correspondingly greater. As the pore becomes wider by longer etching times, the contribution of surface conductance relative to bulk conductance becomes less and less, until finally the measured conductance is approximately equal to the anticipated bulk conductance. The same phenomenon occurs if divalent cations or protons are added, because they bind to the negative charges and neutralise them. Surface conductance also disappears as the ionic strength is increased, because of shielding of the fixed charges by high concentrations of ions, that effectively reduces the Debye length of the fixed charge.

If current through narrow PETP pores is carried largely by cations, then direct measurement of the flow of $K^+$ and $Cl^-$ should reveal a difference in favour of $K^+$. Using radioactively-labelled $Rb^+$ (an analogue of $K^+$) and $Cl^-$, this is exactly what is observed; moreover flow is inhibited as divalent cations are added or the pH is reduced. Such methodology allows one to measure the flow of neutral molecules, like water, glycerol or sugars, also. In this instance, it turns out that flow is not inhibited by divalent cations or protons. This result leads us to conclude that divalent cations and protons do not alter the dimensions of PETP pores and that it is therefore unlikely that fluctuations of conductance between high and low conductance states are due to architectural changes either. In other words, PETP pores do not oscillate between 'open' and 'closed' configurations. To what, then, can the fluctuations be ascribed?

We propose that fluctuations of conductance are due to fluctuations of surface charge: to an ionic phenomenon, not to one involving molecular shape changes. Critics will respond by pointing out that the time scale of ionic changes, such as the protonisation-ionisation of carboxylate groups, is millions of times faster than the changes we observe: they would be unmeasurable in our apparatus. But because of the restrictions imposed by the geometry of the pores, there is intense cooperativity between adjacent charged groups. This leads (a) to much longer dwell times and (b) to far fewer conductance states: a typical PETP pore may contain as many as $10^5$ charges, yet shows only two or so conductance states. We

have constructed a mathematical model based on such cooperativity and it bears out our explanation. Of course if we could measure ionisation changes within PETP pores directly, and show that they fluctuate within a time scale similar to that of ion conductance, it would greatly enhance the validity of our hypothesis. By exposing PETP membranes to a fluorescent cation and measuring spectral changes in a confocal microscope, we have done just that.

First, we note that the fluorescence of a cationic dye, namely a cyanine derivative responds to divalent cations and pH in precisely the manner anticipated on the supposition that the binding of dye is determined predominantly by ionic – and not by hydrophobic – parameters. Next we illuminated a section of the PETP membrane every 3 seconds with a laser beam and measured the resulting fluorescence in the middle of the pore. The result was clear. Individual pores show fluctuations of fluorescence within a time scale of seconds, just like fluctuations of conductance. The fluorescence changes do not reflect 'white noise' or other artefact of the technique, because each pore fluctuates differently. We conclude that conductance changes within PETP pores are indeed due to ionisation changes, and not to an altered geometry of the pore.

To what extent does this explanation for fluctuations in ion conductance apply to endogenous ion channels and toxin-induced pores? We have referred to the fact that physical 'opening' and 'closing' of endogenous ion channels has actually been observed. But such channels also display fluctuations that have appropriately been termed 'open channel noise'. We propose that fluctuations in ionic charge underly this phenomenon. The same explanation probably applies to the fluctuations in conductance of toxin-induced pores that are some 100 times wider than an endogenous ion channel: it is difficult to envisage how structural changes in molecular architecture alone could effectively close a 10 nm wide pore. Indeed we have been able to show directly that conductance changes within a pore created by *Staphylococcus* aureus toxin, for example, are *not* explicable in terms of changes in the effective diameter of the pore.

The experiments with track-etched PETP pores that I have described are significant for another reason. They illustrate the feasibility of mimicking pores through biological membranes with pores through purely synthetic membranes. The technology for the manufacture of 'plastic nerves' is not so far away.

## CONCLUSION

This talk has summarised the knowledge we have gleaned regarding the nature of membrane pores. The journey has taken us from the clinical use of zinc to the measurement of ionic changes in single membrane pores. But I did not travel alone. Without the collaboration of many colleagues, as well as the financial support of different funding agencies, I could not have attained my goal. The fact that our collaborators have come from across the globe testifies to the importance of international cooperation. Since the organisation that I represent – the Oxford

International Biomedical Centre (OIBC) – has recently signed a collaborative agreement with the University of the Philippines, that was ratified by the UP Board of Regents only last month, we anticipate that this new link will benefit the scientific base of both our countries.

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