

Article

Membrane Potential: The Enigma of Ion Pumps

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Abstract: The generation and maintenance of membrane potential is a fundamental part of Membrane Pump Theory. One of the key points of this hypothesis is based on a natural or facilitated molecular diffusion through several types of ion channels and pumps like the Na/K ATPase. Following the principles of chemistry, electrostatics and geometry, it becomes clear that ion channels cannot function in this way. The ions channels cannot by their location have both a filter function and be ion concentrators, and the Na/K pump by its position in the membrane and by the proposed assumptions is not able to perform its regulatory function. The current model must absolutely be revised according to the current state of our knowledge and allow an advance in the understanding of the phenomena opening new research perspectives.

Keywords: membrane pump theory; membrane potential; ion channel; NA/K ATPase; Biophysics; Biology

1. Introduction

This article is the second part of a series on membrane theory and its contradictions.

- Any diffusion? [1]
- **The enigma of ion pumps**
- The Tamagawa experiment
- The ignored shape
- The smoothing of math

In the first paper of the series we demonstrated that simple diffusion or facilitated diffusion could not be a valid hypothesis for the generation of the membrane potential (MP). This second part of the series deals with ion channels and other ion pumps and their behaviour with this diffusion model. Indeed, these protein structures are at the centre of the theory because they would be the "engine" or vector of trans-membrane transport of ions. They would facilitate their diffusion between the interior and exterior of the cell.

The theory would be as follows; The membrane prevents the passage of ions from the outside to the inside of the cell and vice versa. In order for ions diffusion to take place the membrane would contain pores.

These pores are protein structures that facilitate the diffusion of each ionic species between each zone. This is why they are called ion channels.

Each type of ion channel deals with the diffusion of a single ionic species.

There are of course exceptions with exchange channels or pumps. One of the best known is the ATPase Na/K pump which is thought to regulate MP by exchanging 3 sodium ions for 2 potassium ions. This pump therefore removes 3 sodium from the cell and brings in 2 potassium.

The passage of ions through ion channels is subject to discussion: Some think of a hard knocking phenomenon where the ion entering pushes an ion out. The ions would be

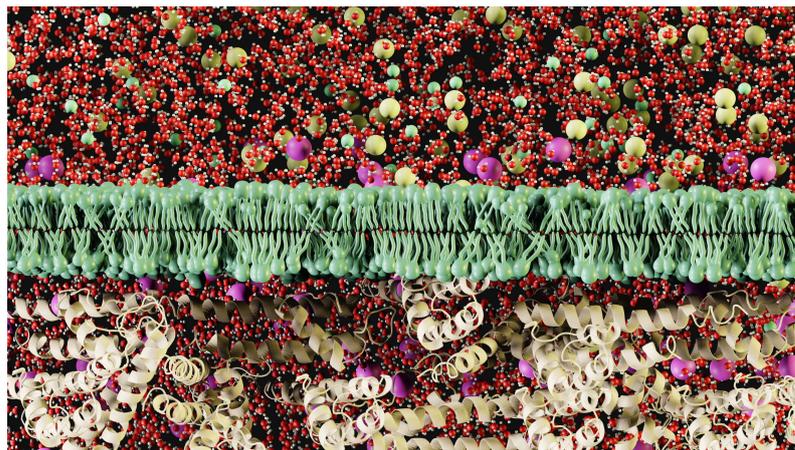


Figure 1. Resting potential as described in the membrane theory. The drawing is simplified in that the amount of water molecules is greatly reduced. The inner compartment, at the bottom, contains the cytoskeleton which is highly proteinaceous and can therefore adsorb ions. It is also shown that the outer part of the membrane has a potassium concentration that is not homogeneous as is the steady state described in the MPT.

aligned in a sequence and in line. Others suggest that it is necessary to make this sequence less hard by interposing a molecule of water between each ion in the sequence. This is called soft knocking.

In addition, it is considered that the ions access the membrane and its channels almost instantaneously. The theory also considers that the ions remain independent in their movements and do not interact with each other. It is also stated that the mobile ions are free and therefore not bound in any way to components of the cell or its membrane.

It is obvious that this theory is based on a series of assumptions that must be verified and that imply a sequence where the slightest grain of sand or contradiction jeopardises the functioning of the system.

The whole theory brings together some extraordinary assumptions, to say the least, and we will show that they are unscientific and open to criticism.

2. Material and Discussion

2.1. The space around the cells

The model imposed on us by theory suggests that the external environment is so vast that it is not (or only minimally) disturbed during trans-membrane ion exchange. We are used to seeing an isolated cell or even a piece of cell surrounded by a liquid ocean which is supposed to represent a nourishing plasma but which remains unperturbed by the small quantities mobilised. This situation is perfectly close to the truth when studying unicellular beings in a natural environment such as a pond or a sea, but is this model still valid when the beings are multi-cellular: does each cell have access to nutrient flows in the same way? What we know about living tissue should leave some doubt. Firstly, a model that works for one cell must also be able to work with many. But, most importantly, it must work, in the same way, to reproduce the conditions found when organisms are arranged in different ways.

We see, for example, in figure 2, which shows a cross-section of unmyelinated axons surrounded by a Schwann cell (pale green) [2], that the peri-axonal space, highlighted by a red colour, is only about twelve micrometers thick. This space is also tenuous for myelinated fibres [3]. Such a small space containing ions cannot have the same ionic dynamics as a cell bathed in a solution that we impose and could impose on us an erroneous vision of the phenomenon.

It is perfectly possible to say, as proponents of the MPT claim, that there is little or no change in concentrations because the quantities involved are so small. But this can be

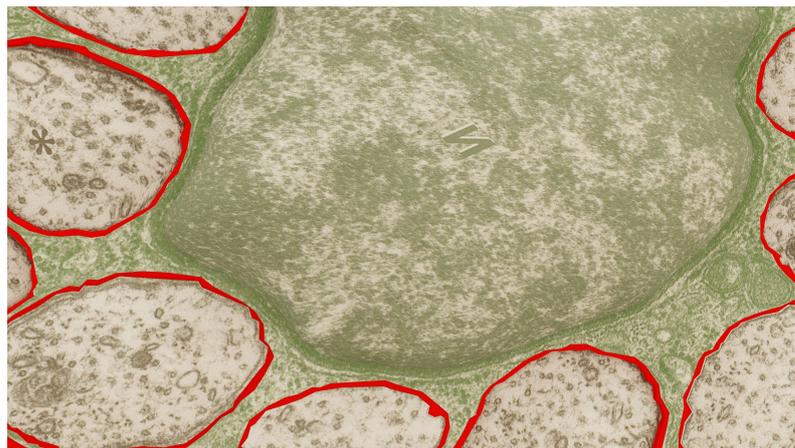


Figure 2. Peri-axonal space in a dorsal root section. The nerve bundle here contains unmyelinated fibres surrounded by a Schwann cell. We have drawn in red the peri-axonal space which measures about 12 nanometers. Original image by Ennio Panesse rights granted to the authors.

rephrased as follows: It is because the quantities involved are very small that there can be no variation with the experimentally imposed quantities. What is important are these small quantities and their variations.

One can use the metaphor of an oil tanker running aground on the Portuguese coast; it is possible to say that the concentration of oil in the Atlantic Ocean is not or only slightly modified, but it would be more judicious to say that the pollution is limited to the Portuguese coasts.

2.2. Empty or almost empty

Unmyelinated fibres contain ion channels in their membranes whose density seems to be related to their diameter. It has been computed that the smallest fibres have 5 ion channels per μm^2 while the largest ones may have 50. We can then compute the peri-axonal volume and its ion content as a function of the ion channel density per μm^2 .

A litre of water is also $1,000 \text{ cm}^3$ or a 10 cm cube ($10 \cdot 10 \cdot 10$). Each of these cubic centimetres can also be represented by a cube with a side length of 10 mm , which therefore contains $1,000 \text{ mm}^3$ ($10 \cdot 10 \cdot 10$). With the same reasoning one can continue this kind of calculation up to the nanometer. $1 \text{ nm}^3 = 1 \cdot 10^{-24} \text{ L}$.

So the volume we are interested in is $(1000 \cdot 1000 \cdot 12)$ or $1.2 \cdot 10^{-17} \text{ L}$.

This surface of $1 \mu\text{m}^2$ must still be divided by the number of ion channels to give us an average surface and above all a side (by calculating the square root of this surface) or distance between two channels. This gives about 447 nm when the density is 5 and 141 nm when it is 50.

It is then possible to see in table 1 that the available quantities are much smaller than those envisaged by Hille [4] and that one must question the relevance of inexhaustible quantities proposed in the current theory. Interestingly, as the density of the ion channels increases, they have less access to ions, again questioning the validity of possible diffusion between the two compartments [1]. These amounts are 10 to 100 times lower than those expected for optimal functioning of these ion channels. Indeed, the notion of steady state becomes irrelevant with such small quantities. The figure 1 shows the complexity of the ionic situation.

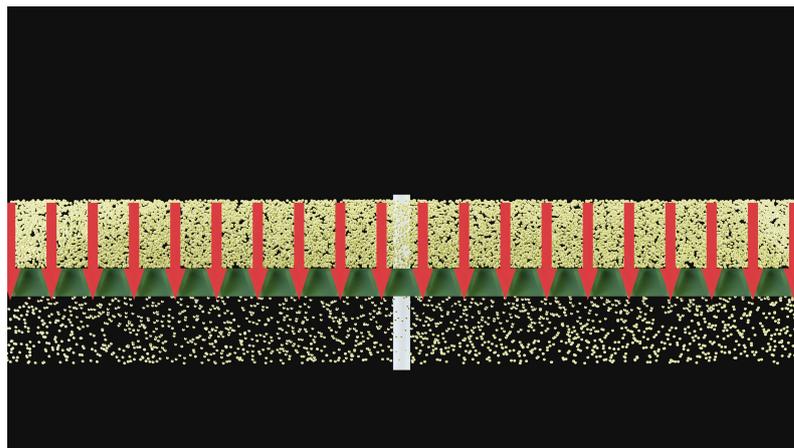


Figure 3. Relationship of the cell membrane to the external (top) and intracellular (bottom) environment. Membrane theory dictates that diffusion occurs perpendicular to the membrane surface. The direction of this diffusion is represented by the red arrows. It is impossible for sodium ions to pass through the ion channel shown in the centre of the diagram in the direction imposed by the diffusion force.

Table 1. Table giving the number of molecules/ions contained in a 12 nm thick volume and accessible to an ion channel as a function of concentration. The first line is for an unmyelinated fibre with 5 channels per μm^2 . The second line is for an unmyelinated fibre with 50 ion channels per μm^2 . Typical values for human concentrations.

$[H_2O] = 55,556$	$[K^+]_{in} = 140$	$[Na^+]_{in} = 15$	$[Cl^-]_{in} = 4$	$[K^+]_{out} = 4$	$[Na^+]_{out} = 145$	$[Cl^-]_{out} = 110$
$8.030 \cdot 10^{+07}$	$2.023 \cdot 10^{+05}$	$2.168 \cdot 10^{+04}$	$5.781 \cdot 10^{+03}$	$5.781 \cdot 10^{+03}$	$2.096 \cdot 10^{+05}$	$1.590 \cdot 10^{+05}$
$8.030 \cdot 10^{+06}$	$2.023 \cdot 10^{+04}$	$2.168 \cdot 10^{+03}$	$5.781 \cdot 10^{+02}$	$5.781 \cdot 10^{+02}$	$2.096 \cdot 10^{+04}$	$1.590 \cdot 10^{+04}$

2.3. The count is not right

Biology is often innovative in its ideas and theories. Permeability has been one of those great ideas that was supposed to solve some of the thorny problems of the generation of the MP and its maintenance [5]. However, this attractive idea and theory is not currently taken up by "traditional" chemistry [6].

This notion is limited to biophysics and biology, but this seems perfectly normal since its use is bound to their fields. The concept of permeability tells us that the membrane is more or less able to let ions or other molecules through. This idea is elegant but poses a problem that seems major: the value is more imposed than deduced from experimentation. A reference value is imposed on one type of ion and the other permeabilities are deduced accordingly.

The case of the Na^+/K^+ pump is much more exciting but also intriguing. According to the authors [7,8], its action is continuous and constant for both the resting potential and the action potential. As we said before, the ion pump takes 3 sodium ions from inside and exchanges them with 2 potassium ions from outside. It should be noted that the ions exchanged all come from areas that contain the least amount of them. And of course, these same ions come by diffusion from the areas where they are most concentrated. The complete cycle of this famous pump is then obvious: to maintain a high concentration of the most concentrated environments, whether in potassium or sodium.

Almost all articles concerning the participation of the Na^+/K^+ pump indicate a relative importance. The participation in the resting potential seems to be limited to around 5%. It is therefore excluded that its action can significantly modify the action potential or its return to normal. The studies where its action is cancelled show only a slight increase in the MP since it is considered negative by convention.

But the count is still not right because theory tells us that the permeability of potassium is 95 times greater than that of sodium for the resting potential.

$$p_{K^+} \gg p_{Na^+} \quad (1)$$

with

$$p_{K^+} = 95, p_{Na^+} = 1, p_{Cl^-} = 2, p_{Ca^{2+}} = 2 \quad (2)$$

We must accept this value or reject it if it is against all logic. Thus, 95 K^+ ions are released when only 1 Na^+ ion enters the cell.

That is 285 K^+ for 3 Na^+ .

We are then also forced to acknowledge that every time the pump runs a cycle, it leaves 283 K^+ ions outside! One could of course assume that the number of pumps is large enough to overcome this obstacle, but simple logic tells us that the number of Na^+ ions available would not be increased. This does not work for the resting potential and it does not work for the AP because the theory does not take into account the permeabilities and ionic concentrations involved.

2.4. A tortuous diffusion

Surprisingly, there are no simulations of the internal or external pathway of the ions to the ion channels. We usually claim that the quantities are such that it does not matter and that, above all, this quantity does not change the direction of the ions in the diffusion process. Otherwise, if the direction of the diffusion is changed, then the theory must also be changed.

Yet most authors are aware of the numbers in the table 1. It is always a good idea to try to represent to ourselves the enormity of these values. It is an excellent way to become aware that there is something wrong with our assumptions and theories.

We have reproduced the sodium ionic conditions on both sides of the cell membrane in Figure 3. The number of ions respects the calculated quantities indicated in table 1. The red arrows represent the direction of diffusion, which must be perpendicular to the membrane. The ion channel is symbolised by a light-coloured cylinder in the centre of the image.

Our brain can only reject the hypothesis and yet the situation shown is much simpler than the one in the theory. It is not possible for the ions to pass through the ion channel without changing their directions, nor is it possible for them to have done so at the same speed or with equal duration. If Cl^- ions had been represented, we would have understood that collisions between cations and anions would prevent the expected ideal diffusion.

If we orient the arrows representing the direction of diffusion towards the ion channel, we obtain a more plausible representation of the phenomenon (see figure 4).

Figure 5 shows the situation from above. It is even more obvious: if diffusion is effective through ion channels then it is neither linear nor unidirectional. Ionic collisions will also be multiplied and diffusion slowed or even prevented.

Another consequence that has not been considered by this topology is that the flow of ions towards the ion channel would experience an increase in its concentration. This kind of hypothesis complicates in a major way, once again, the possibility of diffusion between the two compartments.

2.5. Dumb design

If we take all the ionic movements for the maintaining of the resting potential, we have:

- A massive output of potassium ions.
- A very small amount of this potassium is re-injected into the cell by the Na/K pump.
- A very small amount of sodium enters the cell.
- A very small amount of sodium leaves the cell through the Na/K pump.

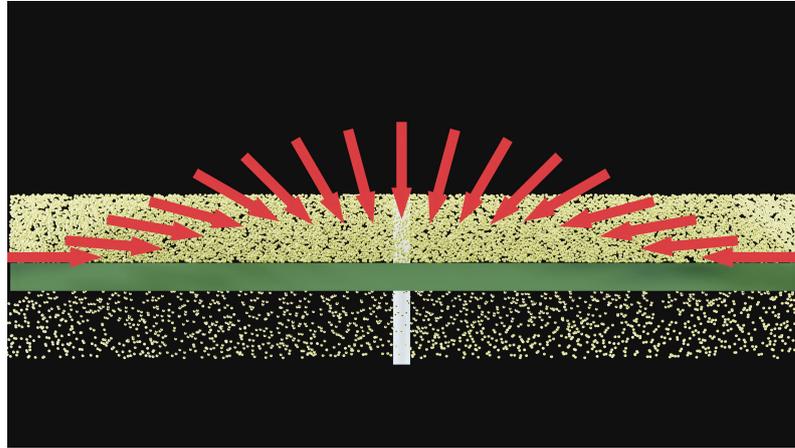


Figure 4. Relationship of the cell membrane to the external (top) and intracellular (bottom) environment. It is more logical to assume that the ions must go towards the ion channel. This disrupts the diffusion and the direction of flow is no longer uniform, nor is it directed towards the membrane.

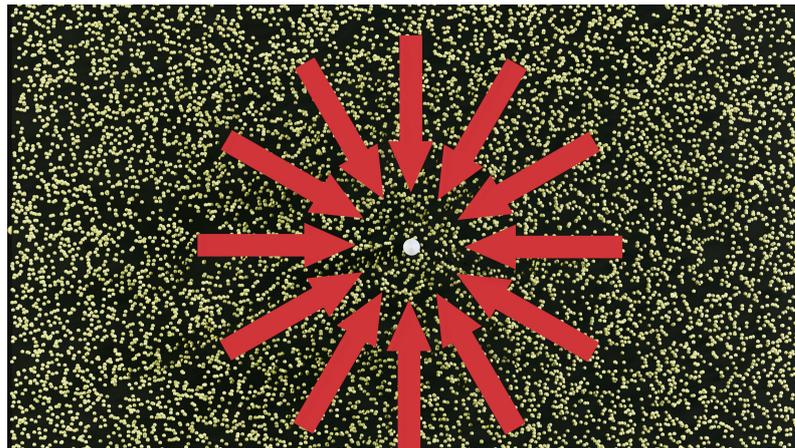


Figure 5. Relationship of the membrane to the ion channel seen from above. It is logical to assume that the ions go to the ion channel. The theory becomes invalid for a uniform diffusion towards the membrane. The situation becomes chaotic if we consider several ion channels, as the flows will then lead to a diffusion that is neither uniform nor linear.

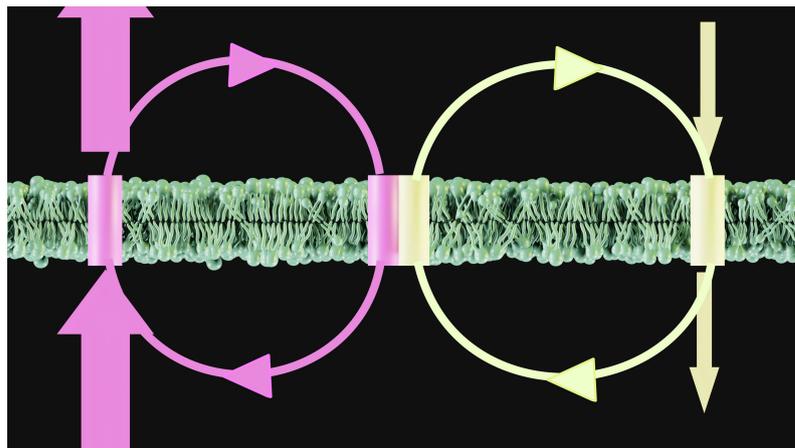


Figure 6. The maintenance of the membrane potential involves ion channels that are located in the membrane. The most involved in this process are the K^+ ion channels (left), the Na^+/K^+ pumps (centre) and the Na^+ channels (right) which are thought to have a primary function in the generation of the action potential. Whatever their position on the membrane, there will be an opposite flow between the different types of ions. Diffusion will be slowed down or even stopped.

This statement, already unbalanced by its quantitative content, becomes inoperative because of the position, in the membrane, of these channels and pumps. Indeed, everything happens in a narrow band of a few nanometres on either side of the membrane.

The figure 6 shows visually the principle of maintaining the membrane potential or resting potential for the neuron. This figure, limited to a single element of the system, shows ionic circulations in opposite directions which cannot achieve the desired effect.

Therefore, it will never be possible for this process to have any effect on the concentration of the different types of ions contained in the external environment or in the cell. The expected regulation process is not achieved due to the wrong location of the components in the system.

2.6. Hard? Soft ? or rather null knock

Figure 7 is a kind of reconstruction from an early paper on the K^+ ion channel [9]. This static picture imposes drastic initial conditions. If we are shown this image it is because it is part of a hypothesis that we think is viable.

It is often stated that ion channels do not act as filters or sieves. Yet at the same time we accepted that the ions are hydrated and therefore surrounded by layers of water molecules. And we also accept that they lose these water molecules when they enter the ion channel pore. There is indeed an ionic concentration in the ion channel that is not considered in the theory or hypotheses. Moreover, there is a selection of ions among several types from a "soup" that should be homogeneous but could not be.

It is therefore not sufficient to say that the ions are dehydrated when they pass through the ion channel. It is also necessary to explain by what means and also to deal with the molecules that are left behind and the disturbances and restructuring that may occur in the solution.

The structural study of ion channels often requires a crystallisation technique that freezes the various proteins that make up the channel. This fixation does not guarantee that this conformation is always identical at operating temperature. Moreover, the position of the ions inside the pore is almost always a matter of simulation. It should be remembered that this simulation assumes that the ions enter the pore by diffusion. The researchers do not agree with the type of ion propagation within the pore. There is still dissension between hard and soft knocking [10–13].

The vast majority of the latter cannot ignore the fact that electrostatic coulombic interactions are the most important at this scale. By simply drawing a first level of these

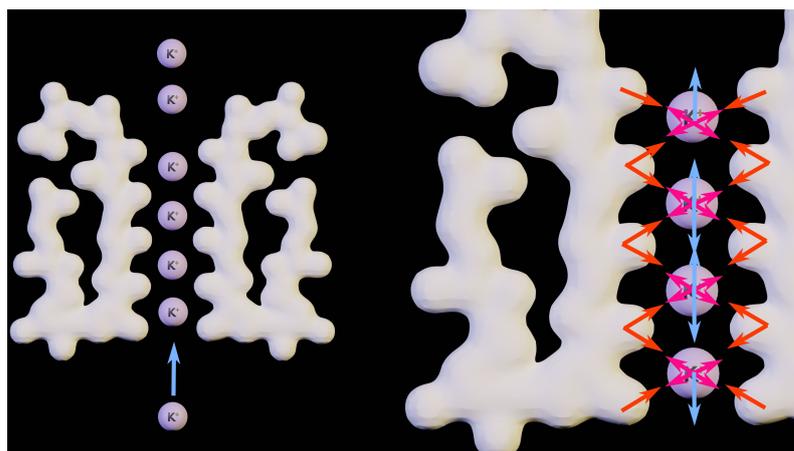


Figure 7. A "hard knock" K^+ ion channel. Image often shown in articles to illustrate a K^+ ion channel in action. This snapshot would suggest that the ions come in single file, shedding the hydration layers of water molecules before entering the pore. Each incoming K^+ ion would then be able to push an ion present in the pore in the manner of a Newton's cradle. The right-hand side shows a first level of electrostatic interactions between the ions and the walls of the ion channel. Half of the walls are shown.

interactions on the ion channel, as shown in figure 7, we obtain a conclusion without any contradiction. The presence of two or more ions in the pore causes a mutual repulsion that would drive them to be ejected from the channel in the opposite direction, but they are also held together by the charges present in the pore walls.

It is also argued that the presence of a "foreign" ion, for example sodium, would cause the channel to narrow and malfunction. This blockage would then be permanent and is unlikely to occur [14]. It must be reiterated that the diffusion force is weak compared to the electrostatic forces present. How could an ion move forward in the pore when the forces that will hold it in place? Worse, if the channel empties then the forces will pull its edges apart by the same electrostatic forces.

And if the ion is present at the entrance of the pore, the pore would tend to close up, preventing its effective input. The charges of the wall are attracted more and more as the distance between the ion and the pore decreases. Although we are now beginning to think that the elements of the ion channel are mobile, we rarely take this mobility into account in simulations.

From any point of view, the operation of the canal is not only hazardous but utopian. Nature has accustomed us to more simplicity, logic and efficiency. There is no lack of studies showing alternatives that are not only plausible but also respect the amount of energy available in the cell [15–20] and also [21–26]. Adsorption has been a recognised process in chemistry for decades but there is a kind of resistance to its "entry" into biophysics and biology. This seems normal because it challenges many theories but isn't science always questioning?

3. Conclusions

We saw in the first article that diffusion cannot be a force in the generation of the membrane potential. This article further aggravates this possibility by raising more than justified doubts about the filtering and selective function of ion channels.

It has been shown that the Na^+ / K^+ pump is completely inefficient due to its disadvantageous position in the membrane. It is shown that the ion channel in its theory cannot function as a selective "diffuser". It is also shown that diffusion cannot have the direction imposed in the equations used to compute the membrane potential.

If diffusion cannot operate and ion channels cannot function, the two can still be combined. Proteins are perfect sites for attracting ions and ions make these proteins mobile

and dynamic. That these proteins are not true ion channels opens up other possibilities that are more respectful of the science we already have at our disposal.

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