Voltaic Properties of a Novel Channel Protein: Triplin

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Introduction

In Spring of 2021, I started working under Dr. Marco Colombini's lab and partook in his experiments dealing with the novel cannel porin: triplin. In the lab I was tasked with the formation of the phospholipid bilayer, insertion of the protein channel into the created bilayer, and analysis of data collected from applying voltages to the successfully formed membrane-protein complex.

Site Information:

Name of Site: University of Maryland Biology-Psychology Building

Address: University of Maryland, College Park, Maryland 20742

Your supervisor: Dr. Marco Colombini

The site mission: "The broad objective of Dr. Colombini's research is to understand how the molecular machines, that drive the life processes in cells, work. An important unanswered question under current active investigation by Dr. Colombini, his collaborators, and his students is the molecular mechanism underlying the voltage control of membrane channels."

The particular goals of the site you were at: My particular goals at the site are to gather information around the electrophysiological characteristics of the novel channel porin triplin, in order to further expand the knowledge around its cooperativity and voltage dependence. This all further contributes to Dr. Colombini's mission to understand how life is able to become living.

Methods – Electrophysiological Recordings & Theory and Calculations

- A planar phospholipid membrane is formed by the monolayer method
- Triplin was then reconstituted into the membrane and studied under voltage-clamp conditions by applying a triangle wave of voltage
- The Boltzmann distribution was used to determine the parameters of voltage dependence

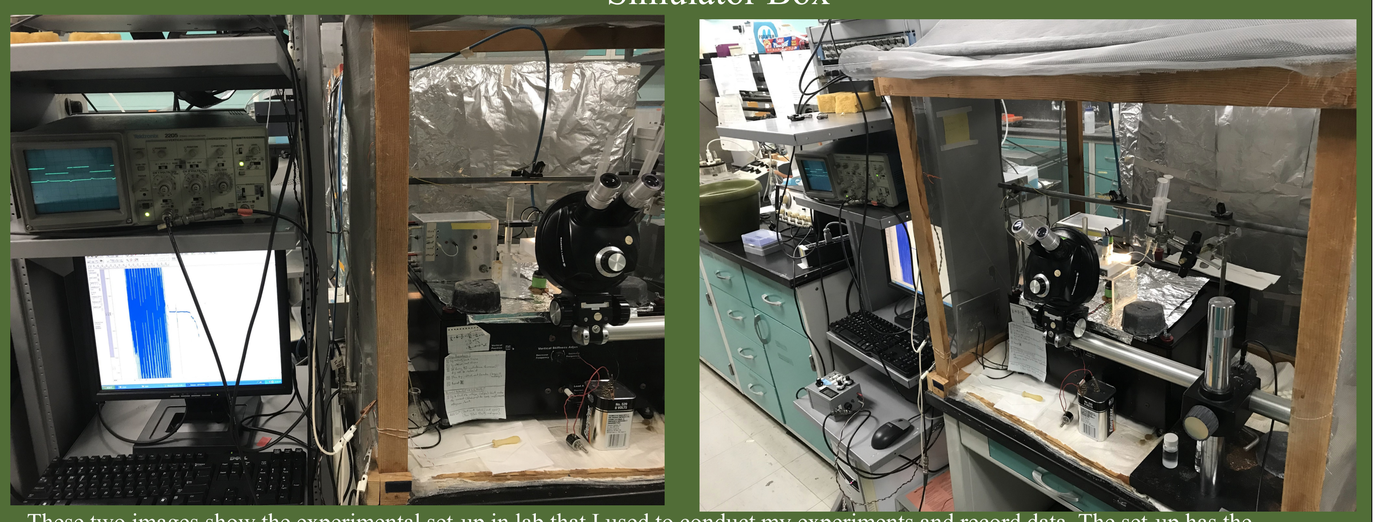
$$\frac{P_O}{P_C} = e^{-\frac{\Delta E}{RT}}.$$

$$\Delta E = nFV_0 - nFV = nF(V - V_0)$$

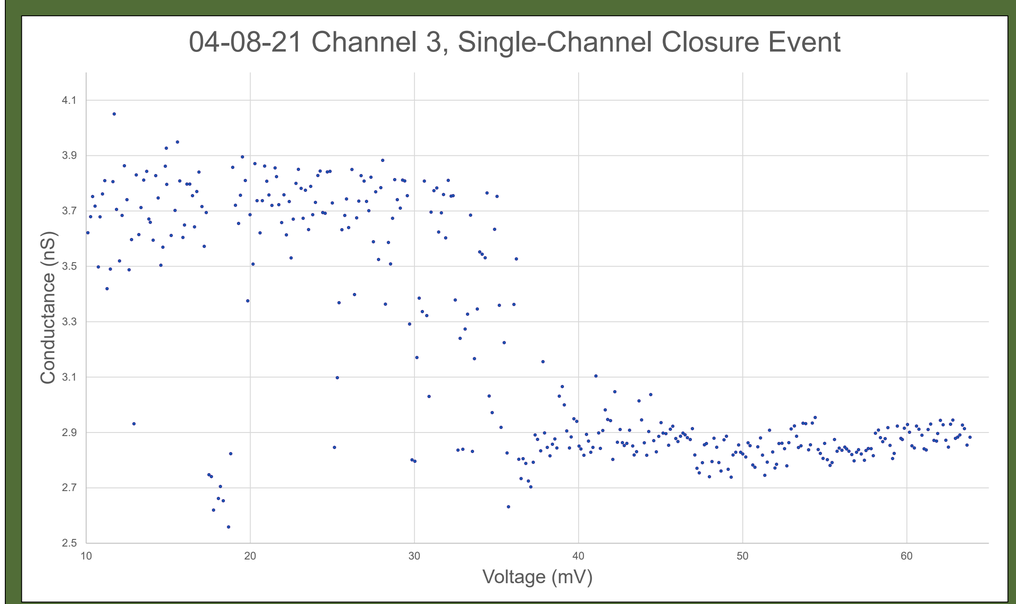
$$\ln\left(\frac{G_{max} - G}{G - G_{min}}\right) = \frac{nF}{RT}(V - V_0),$$

Materials

- Teflon chamber composed of 2 half-chambers with a Saran partition in between them
- Microscope
- 2 10 ml Syringes
- Computer & Chart Recorder
- 2 electrodes filled with saturated KCl solution inside
- Electric motor
- Amplifier
- Oscilloscope
- Simulator Box
- HEPES/HCl salt solution
- Phospholipid solution
- 5% Petrolatum Solution
- Triplin Protein



These two images show the experimental set-up in lab that I used to conduct my experiments and record data. The set-up has the Teflon chamber in the middle, with the syringes and electrodes in the salt solution, and an electric motor below the chamber. The electrodes are attached to the amplifier, which is attached to the simulator box which determines the voltage applied to the chamber.



Triplin is made of 3 channels , with channel 1 & 3 responding to positive potentials and channel 2 responding to negative channels, with all three channels showing a form of cooperativity in their closures and openings. This graphs shows the event of a closure of the third channel in a triplin protein, which can be denoted by the drop in conductance at a high positive potential. This also shows the oscillatory nature of the closure of channel 3

Results and Discussion:

The experiments that I conduct take a lot of time and although results are obtained, interpretation of those results are usually done after many repetitions, so there can't be any major claims from the results I have gathered. From what I have gathered now, the results show the general trends that have been seen with triplin: channels 1 and 3 close at negative potentials and channel 2 closes at a positive potential. Current experiments are done to analyze the effects of introducing anhydride to the chambers after successful insertion of the triplin protein was made into the formed membrane. The anhydride hasn't shown consistent enough results to make a definite claim, but a couple of experiments have shown that introduction of the anhydride limits or halts the oscillatory nature of the closings of the channels. These observations may aid a model described by the lab team which states that there are to mechanisms in which triplin closes its channels: one allowing the channel to bounce back and forth between opened and closed (oscillations) and one not allowing the channel to have an oscillatory nature.

Acknowledgments:

I would like to thank Dr. Colombini immensely for his efforts in broadening my critical thinking abilities and guiding me throughout this experience. I would also like to thank Dr. Holtz and Dr. Merck for all the effort they have put into this program in order to broaden all of our minds and interests.

Bibliography:

1. Colombini, M., Lin, S., Chang, K., Cherian, N., Wu, B., Phee, H., Cho, C. 11 September 2019. Cooperativity and Steep Voltage Dependence in a Bacterial Channel https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6770917/ (accessed Apr 16, 2021).