3-28-2014

Analyzing the Efficiency of Inserting GP10 into a Planar Bilayer Membrane Dopped with a Carboxylated Lipid

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Abstract

The phi29 connector, GP10, is a channel protein from the bacteriophage phi29, a virus that can infect bacteria. The protein provides a channel that allows the phi29 genomic DNA to go through it during the infection process in-vivo. It has been successfully demonstrated that the connector can be inserted into a planar lipid bilayer membrane in-vitro (Wendell, et al, 2009), and thus, the channel protein could potentially be used as a single-molecule platform to detect individual molecules (both small molecules and macromolecules), to monitor chemical and biochemical reactions in real time, and to perform DNA/RNA sequencing. Understanding the process of inserting the protein translocation through the planar bilayer membranes will allow scientists to design a platform for the novel biosensor (Moleiro, et al, 2012). However, the current problem encountered is the difficulty in achieving stable and efficient insertions of the protein into a synthetic lipid, 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC).

Objectives

We will compare differences between the three particular factors that the addition of the connector in the lipid would give: the insertion efficiency, which are the total amount of insertions per sample time; the time of each insertion; and the total amount of insertions per sample batch. Furthermore, we will employ an independent two-tailed t-test to determine whether the differences are significant, thereby implying if the modified carboxylated lipid molecule does indeed stabilize the channel.

Methods

Using molecular biology methods to prepare the protein GP10, we employed a new strategy to optimize the lipid for efficient orthogonal integration of the phi29 connector into bilayer lipid membranes. Utilizing the precise knowledge of the 3-D structure of the phi29 connector, we optimized the bilayer membrane by tuning reactivity with the help of the DPhPC dopped with a carboxylated lipid, N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1-Palmtoyl-2-azelaoyl-sn-glyreco-3-phosphocholine (PaPC), as seen in Figures 1 and 2.

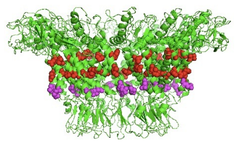
Conditions

- Buffer
  - 1 M NaCl
  - 0.5 M Tris
  - 2 mL in bottom half of chamber
  - 0.5 mL in top half of chamber
- pH: 8.0
- Voltage applied: -40 mV
- Volume of Protein inserted:1 microliter
- Teflon membrane

Results

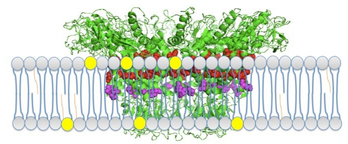
Initial results do confirm that the initial problem lies in the stabilization of the synthetic lipid. Furthermore, the efficiency of the protein insertion is great affected by the stabilization of the lipid. Figures 3 and 4 indicate the number of insertions that each lipid has given thus far. Figure 5 shows that the unmodified lipid produced only 3 insertions in 20 minutes, while the carboxylated lipid in Figure 6 produced more than 4 times the amount of insertions that the unmodified lipid gave in the same amount of time. In Figure 6, the carboxylated lipid is more stable than the unmodified lipid in Figure 5.

Figure 1: Carboxylated phi29 Connector



The carboxylated connector contains positively charged Arg17 (top red), Arg22 (middle red), and Lys172 (magenta) that would theoretically increase interaction between the positively charged connector and the negatively charged protein.

Figure 2: Carboxylated phi29 Connector Protein in Lipid Bilayer



The lipid bilayer is formed from DOPC (white heads) mixed with PAcPC (orange tails), and the anionic lipid POPG (yellow heads). The POPG gives the membrane a net negative charge which will then interact with the positively charged Arg and Lys groups, allowing for the connector to be inserted into the lipid bilayer membrane.

Figure 3: Current Jump Distribution of Unmodified Lipid
Dates: July 16, 2013 - February 24, 2014
Total Data Points: 100

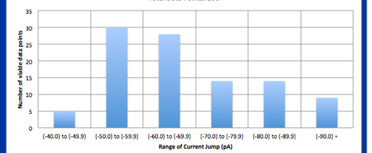


Figure 4: Current Jump Distribution of Carboxylated Lipid
Dates: February 28, 2014 - March 7, 2014
Total Data Points: 55

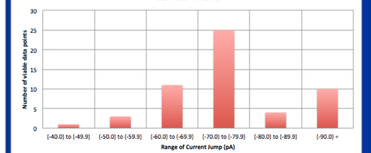


Figure 5: Unmodified Lipid Insertion Example

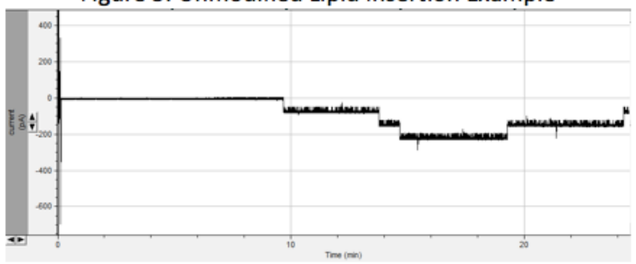
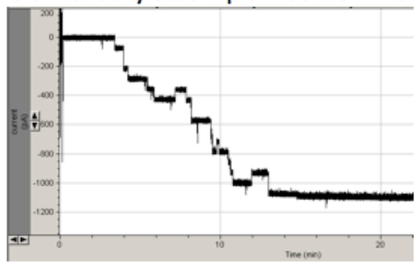


Figure 6: Carboxylated Lipid Insertion Example



Conclusion

Although the small sample of data may imply that the difference between the two kinds of lipid is significant, more data points must be acquired. Also, an independent two-tailed t-test will be performed to verify the significance of the data. Nevertheless, the fact that the carboxylated lipid provided more insertions than the unmodified lipid shows that this method may be more efficient in obtaining protein insertions.

References

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Acknowledgements

I would like to thank Dr. Peng Jing from the Department of Chemistry for his insight with this project. I also would like to thank Josiah Gerber, an IPFW student, for his work in creating the protein GP10.