

# Electrophysiological Characterization of $\alpha$ -Hemolysin Nanopores in Lipid Membranes

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**Abstract** We measured the current flow through water-filled nanometer-scale pores formed by  $\alpha$ -hemolysin ( $\alpha$ -HL) in lipid bilayer membranes. Wild type  $\alpha$ -HL made in our lab this summer was compared with a standard obtained from NIST. Additionally, eight  $\alpha$ -HL mutants were made and fluorescently tagged with two fluorescent labels in our lab. We tested the ability of these mutants to function like the wild type in nanopore formation with and without a label. All eight mutants were functional. Five mutants with fluorescent labels were tested and were also functional. Based on these findings we will proceed with using single-molecule optics to study the nanopore formation with the fluorescently labeled  $\alpha$ -HL.

## Introduction

The protein  $\alpha$ -hemolysin ( $\alpha$ -HL) creates toxic heptameric nanopores in cell membranes. These nanopores can be replicated on a lipid bilayer membrane for experimental purposes. A lipid bilayer acts as a capacitor in an ion-rich solution. A voltage can be applied across a membrane and when a nanopore inserts into the membrane, a small amount of current passes through. The increase and decrease in current when a channel opens and closes occur in discrete steps. The protein can be characterized by the amount of current that passes through the nanopores it forms. The number of channels in the membrane can be controlled by altering the amount of protein added to the membrane. At low concentrations, a single channel will insert in the membrane. The polarity of the applied voltage can be reversed and the nanopores will conduct greater current at one polarity than the other. The degree to which  $\alpha$ -HL preferentially conducts in one direction is called the rectification ratio and is another way that the nanopores can be characterized.

Eight mutants were made that can be labeled with fluorescent tags and two different labels were attached to each of the mutants. We needed to test the functionality of the labeled mutant  $\alpha$ -HL to see if either the mutation or the fluorescent label would inhibit the formation of the nanopores.

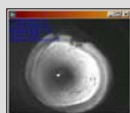


Figure 1. A 50  $\mu$ m aperture (5X)

## Experimental Methods

### 1. Sample Chamber Setup

- The tip of a pin, when pushed through PTFE tubing, creates a 50  $\mu$ m tapered aperture
- The aperture is put into the sample chamber

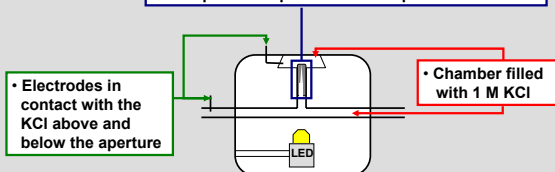


Figure 2. Setup of the sample chamber.

### 2. Membrane Formation

- Lipid/pentane mixture applied to the aperture and allowed to dry
- Sample chamber filled with 1M KCl
- Lipid/hexadecane mixture brushed over the aperture
- Air bubble is passed over the aperture via an empty micropipette
- The air bubble is slowly removed and a lipid membrane spontaneously forms

### 3. Nanopore Formation/Data Collection

- 0.5  $\mu$ L of  $\alpha$ -HL in 2 M KCl is placed over membrane
- Current flows between the electrodes when channels insert in the membrane
- A discrete current change is seen when a channel opens or closes
- The current is amplified, filtered and recorded on an oscilloscope
- This was repeated with each of the eight mutants: unlabeled, labeled with the fluorescent tag TMRIA, and labeled with the fluorescent tag BODIPY, and both of the wild types for a total of 26 materials

### 4. Data Analysis

#### Step Size

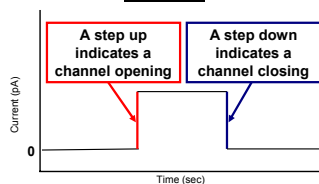


Figure 3. A discrete increase in current indicates the opening of a channel. Likewise, a discrete decrease in current indicates the closing of a channel.

#### Rectification Ratio

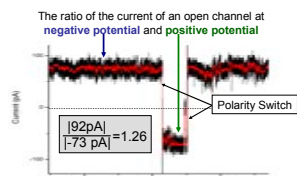


Figure 4. Current through one open channel versus time. The red lines indicate 25 point adjacent averaging.

#### Channel Stability

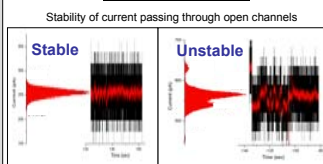


Figure 5. All points histograms of a 25 point adjacent average of the current (red) shown with the current data. A stable current through open channels (left) and an unstable current through open channels (right).

## Results

- All eight of the mutants were functional
  - $\Delta$ 69 was the only mutant that appears to have been adversely affected by the mutation
- Two mutants were tested with TMRIA and BODIPY and all were active and functionally similar to the wild type
- Three additional mutants were tested with one of the two labels and all were active and functionally similar to the wild type
- The  $\Delta$ 244 BODIPY mutant is currently the most well-characterized mutant, showing little difference from the wild type
- ANOVA shows that the rectification ratios of our wild type, the NIST wild type and each of the mutants tested are statistically identical
- The means for the step up and step down size of the NIST wild type, our wild type and the  $\Delta$ 244 BODIPY mutant were similar (Figure 6)

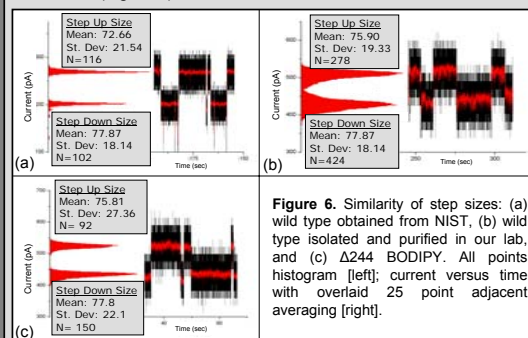


Figure 6. Similarity of step sizes: (a) wild type obtained from NIST, (b) wild type isolated and purified in our lab, and (c)  $\Delta$ 244 BODIPY. All points histogram [left]; current versus time with overlaid 25 point adjacent averaging [right].

- Instability was observed in some of the mutant channels but there is not yet sufficient data for full analysis

## Conclusions/Ongoing Study

- To date, all materials tested (16 of 26) demonstrate channel forming activity
- Statistical comparison shows that mutation and subsequent labeling of the protein do not impair the functionality of nanopore formation in seven of the mutants
- The adverse affect of the  $\Delta$ 69 mutation was not surprising given the location of the mutation
- Complete the characterization of all 26 materials
- Begin single-molecule optical studies to directly visualize the nanopore formation process