Investigating the Effect of Charge Distribution on Membrane-Active Antimicrobial Peptides
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Antibiotic resistance furthers the need for novel therapeutics. The widespread use of antibiotics has allowed pathogens the time and opportunity to evolve resistance to these treatments, and the implications are striking. In 2019, there were an estimated one million deaths (globally) caused by antibiotic resistance, and over five million associated with antibiotic resistance.[1] Thus, new avenues of drug development are imperative to explore. Antimicrobial peptides (AMPs) defend many classes of organisms from pathogens and can be specific to certain areas of infection.[2] These peptides are short sequences of amino acid building blocks and can adopt various structures. AMPs are bactericidal, killing pathogenic cells, rather than simply halting the further proliferation of harmful cells, placing AMPs in a promising position for being an effective treatment option.[3] Contrary to common antibiotics, AMPs are especially encouraging because they directly and selectively interact with and disrupt membranes, which are evolutionarily stable. This means that pathogens will have a much more difficult time changing their membranes as a response of resistance to this possible antibiotic.[4]

The mechanism of membrane disruption by AMPs follows a general two-step sequence. First, the peptides bind at the water-membrane interface, and secondly, at a certain concentration of peptide, the AMPs can insert themselves into the membrane. This insertion causes distortion of the membrane and can eventually lead to pore formation, effectively killing the cell.[5] Two models of pore formation, barrel-stave and toroidal (Figure 1) have been proposed, and they differ in ways that can be experimentally studied. Toroidal pores are essentially more disruptive and can be more effective at killing cells.

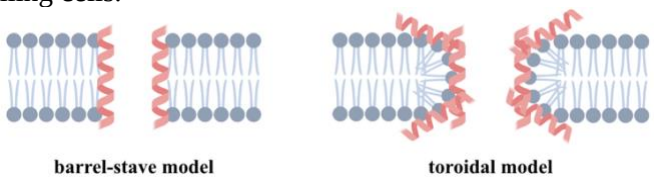

Figure 1. Models of pore formation by antimicrobial peptides (AMPs). Red helices represent AMPs, and the blue objects represent the membrane.

My project focuses on investigating the effects of modifying a particular AMP, alamethicin (ALM) on which a barrel-stave type of pore is formed. A simulation found that naturally occurring ALM forms barrel-stave pores, whereas a mutated ALM with an added charge forms more toroidal-like pores.[4] I can experimentally study pore formation and pore dynamics through observing dye leakage from vesicles, ultimately eliciting what type of pore is formed from these data because different pore types show different kinetics and leakage profiles. I used different fluorescent dyes to label the membrane in red and to fill the inside of the vesicles with green. These are visualized by fluorescence microscopy, where, shining a specific type of light selectively illuminates these differently colored dyes.

I first optimized the process of forming the vesicles needed for these dye leakage studies. The formation process includes applying an alternating electric current to the sample to create vesicles large enough to be observed by optical microscopy, so I looked at different procedures and systematically changed different parameters (voltage and voltage gradient, duration, frequency). I monitored vesicle size and useability (via confocal and fluorescence microscopy) that were produced by these methods. I found that an optimal formation parameter consisted of a constant 5 volts and 10 Hertz over one hour, which was easier and less timely than other methods, without sacrificing vesicle size or useability.

The second part of my project focused on creating the physical system needed to perform these experiments. I used the principles of soft lithography, the process of using molds and elastomeric materials to create microscale systems, which started with designing and 3D printing a mold. I then formed the channels in which I will perform my dye leakage studies. I poured a transparent, chemically inert material into the molds, cured the material with heat, and then attached the cured channel onto a glass microscope slide. The next steps of this process include introducing the dye-filled vesicles into the channels, immobilizing them, introducing the AMP, and finally monitoring the leakage of dye form the vesicles as AMPs make pores in the membrane.

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