

## Shining a Light on Antimicrobial Peptides: The Influence of Charge Distribution on Alamethicin Pore Dynamics

Rhys Edwards, 2025

While the development of traditional antibiotics slows, bacteria develop resistance to our antibacterial arsenal faster than ever, causing over 200,000 deaths in the past year alone. To counter this imminent threat, the scientific community has turned their focus towards antimicrobial peptides (AMPs): short strings of amino-acid building blocks with antimicrobial properties. AMPs' advantage over traditional antibiotics comes from their ability to target and destroy bacterial cells by forming hole in their membranes. Since the membrane is largely full of non-genetically encoded materials like lipids, future generations of bacteria cannot develop resistance to the AMP. These holes in the membrane can be different in structure, with toroidal pores that bend the bilayer membrane being more effective in killing bacteria than its counterpart, the barrel-stave pore, that causes no membrane deformation.

Zemel and his team hypothesized that the distribution of charge along AMPs influences the type of pore formed.<sup>1</sup> This hypothesis was later supported by another team with a molecular dynamics computer simulation which showed that adding a positive charge in the middle of the AMP Alamethicin changes its pore from barrel-stave to toroidal.<sup>2</sup> However, computer simulations cannot encompass the vast complexity of real biological and chemical systems. Therefore, my research seeks to examine how charge distribution affects AMPs and their respective pore structures in a more realistic environment.

As these pores are too small to see with a confocal microscope, a more indirect method of observation must be employed. While ATR-FTIR is an analytic technique mainly used to identify the chemical contents of a sample by using infrared light to excite specific vibrational states unique to the sample's chemical structure, it can also be used to determine any 3D structure within the sample. This methodology is particularly useful for our research, as the barrel-stave and toroidal pores differ in the respective tilt of the peptides (and thus the 3D structure) within the pore. If we create a supported membrane on the ATR-FTIR surface crystal and induce AMP pore formation within the membrane, we can preferentially excite the same amide bond on the AMP differently depending on the angle of polarization of incoming light. This will allow us to interpolate the angle of the peptide within the membrane as a function of the measured intensity ratio.

Over this past summer, I have been developing a procedure to form membrane bilayer structures reliably on the ATR-FTIR crystal surface. My initial procedure involves the synthesis of large (~100 nm) spherical lipid structures (vesicles). These are made by suspending lipid in a buffer solution, and then using a freeze-thaw method followed by extrusion to assure uniformity. The vesicles are then deposited onto the surface of the ZnSe crystal where they should rupture to form a continuous bilayer. However, my results indicate that this technique results in the formation of a vesicle layer as the vesicles are not rupturing. To induce rupture, I then developed methodology to increase the vesicle's affinity for the crystal surface and to decrease the size of the vesicles. I found that cleaning the surface of the crystal with plasma increases the hydrophilicity and vesicle affinity of the surface, and that larger vesicles could be broken down into smaller vesicles through sonication which uses sound waves to disrupt the membranes. This size difference was confirmed through flow cytometry. Using the increased vesicle affinity and smaller vesicle size, I repeated the previous experiments, finding successful lipid bilayer formation. These results were confirmed with the polarizer experiment, as different polarizations of our sample yielded different intensities, characteristic of a bilayer, differing from the previous vesicle layer which would show equal intensities regardless of the polarization due to the symmetry of the spherical vesicles.

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