

**419-Pos****Mechanism of Action of pH-Triggered, Membrane Active Peptides**

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<sup>1</sup>Dept Molec Biophys, Johns Hopkins Univ, Baltimore, MD, USA, <sup>2</sup>Univ Missouri, Columbia, MO, USA, <sup>3</sup>Dept Phys/Biochem, Univ Missouri, Columbia, MO, USA, <sup>4</sup>Dept Biochem, Tulane Sch Med, New Orleans, LA, USA, <sup>5</sup>Dept Matl Sci/Eng, Johns Hopkins Univ, Baltimore, MD, USA. The plasma membrane insulates a cell from its outside environment, serving as a selectively impermeable barrier across which only small or hydrophobic molecules can pass. Although the membrane is necessary for life, it is also problematic when useful macromolecules such as antibodies, peptides, polysaccharides, and imaging agents are blocked from entry. Most macromolecules can easily be up-taken by the cell through endocytosis, but remain trapped and eventually degraded within endosomes, which mature into lysosomes. To promote the escape of macromolecules from endosomes prior to their maturation into lysosomes, we used a high throughput screen to discover pH triggered, pore-forming peptides[1]. To determine their mechanism of action, we measured the peptides' membrane binding affinity, secondary structure, and induction of pore-formation in POPC membranes. We identified that at least 5 acidic residues are essential for mediating a change from a soluble, predominantly unfolded, and inactive state at pH 7 to a membrane bound, helical, and active state at pH 5. These peptides are highly potent, with significant macromolecular leakage occurring at concentrations as low as 2 peptides per 1000 lipids. We determined that the peptides behave dynamically, associating with and dissociating from membranes, and consequently form pores on multiple vesicles. By atomic force microscopy imaging, we confirmed that the peptides form macromolecular pores, with diameters as large as 50 nm. Furthermore, we developed a kinetic model to explain how a low net fraction of bound peptide can lead to significant leakage. This work yields biophysical insights that will improve the design of peptides for new biomedical applications.[1]Wiedman, G., Kim, S.Y., Zapata-Mercado, E., Wimley, W.C. and Hristova, K., 2017. *J. Am. Chem. Soc.*, 139(2), pp.937-945.

**420-Pos****Discovering Novel Antimicrobial Peptides using High-Throughput Screening and Rational Variation**

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Antimicrobial peptides (AMPs) have long been attractive drug candidates for the next generation of clinical antibiotics due to their potent antimicrobial activity and low propensity for inducing resistance in pathogens. However, due in part to toxicity concerns and activity loss in vivo, AMPs have yet to have any impact clinically. Our lab hypothesized that the presence of host cells could cause depletion of free peptide available to target bacterial cells and showed this to be true for some AMPs using concentrated human red blood cells (RBCs) as a model eukaryotic cell. To solve this problem, we synthesized a combinatorial peptide library based on the potent AMP, ARVA, and screened the library for activity in the presence of concentrated RBCs. We isolated nine unique, but similar sequences from the screen. We designed a consensus sequence based on the nine peptides and synthesized it using only D-isomer amino acids to form D-NOGCON. D-NOGCON displays excellent antimicrobial activity against multiple human pathogens in the presence and absence of concentrated RBCs, causes very little hemolysis, and is not susceptible to cleavage by cellular or plasma proteases. D-NOGCON also has high activity against bacterial biofilms and does not readily induce leakage. In this work, we created rational variants of D-NOGCON with truncations, insertions and mutations to test various hypotheses about the basis for highly potent antimicrobial activity with low hemolysis and low toxicity against nucleated cells. In the near future, we will use this information to design a next generation combinatorial peptide library based on D-NOGCON that will be screened for i) clinically relevant antimicrobial activity in the presence of serum and concentrated red blood cells, ii) negligible hemolysis, and iii) negligible toxicity against nucleated cells.

**421-Pos****Toxicity and Structure of Antimicrobial Peptides Derived from the Chemokine, CXCL10**

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Antibiotic-resistant bacteria are a rising public health concern. To counter this threat, new antibiotics need to be developed. Chemokines are a class of im-

mune signaling proteins able to induce chemotaxis of immune cells to the site of infection. In addition to signaling functions, the chemokine CXCL10 exhibits direct antimicrobial activity. This project's goal is to elucidate the mechanism by which CXCL10 kills bacteria and create one or several new antibiotics derived from CXCL10 by optimizing this antimicrobial behavior. To discover the molecular determinants responsible for antimicrobial activity, our collaborative group synthesized a peptide library derived from CXCL10 and screened these peptides for bactericidal activity. Peptides found to comprise broad-spectrum antimicrobial activity represented the N- and C-terminal regions of CXCL10. These peptides were selected for further studies, along with a non-antimicrobial peptide as a negative control. As other antimicrobial peptides are known to form an alpha helix, we used circular dichroism spectroscopy to assay the ability of the three CXCL10-derived peptides to adopt a similar conformation in the presence of a Gram-negative bacterial membrane mimetic. Surprisingly, while both antimicrobial peptides became more ordered in the presence of the bacterial membrane mimetic, only the spectra of the C-terminal peptide showed features of an alpha helix. To determine the potential compatibility of these peptides with human cells, in hopes of developing a novel antimicrobial therapeutic, we assessed the ability of the peptides to lyse human red blood cells via a hemolysis assay. Even at concentrations many fold higher than those required for bacterial killing, CXCL10-derived peptides showed minimal, statistically insignificant hemolysis. These observations are encouraging for further development of antimicrobial therapeutics based on CXCL10.

**422-Pos****Characterization of a Histidine Containing Antimicrobial Peptide with pH Dependent Activity**

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Antimicrobial peptides (AMPs) have been an area of great interest, due to the high selectivity of these molecules toward bacterial targets over host cells and the limited development of bacterial resistance to these molecules throughout evolution. The peptides are known to selectively bind to bacterial cell surfaces through electrostatic interactions as the cell surface is net negative charged while the peptides are positively charged. Once bound, the peptides insert into the cell membrane and cause local disruptions of membrane integrity leading to cell death. Previous experiments showed that when Histidine was incorporated into the peptide C18G it lost all antimicrobial activity. Due to the side chain pKa is near physiological pH, we wanted to investigate the role of pH on the activity of the peptide. MIC results demonstrated that decreased media pH increased antimicrobial activity. Intrinsic Trp fluorescence was used to perform binding assays to model lipid vesicles under different pH conditions while Trp quenching was used as a reporter of the local environment. TCE quenching was used to determine peptide aggregation state in solution and showed a clear pH dependence on peptide aggregation. Acrylamide quenching demonstrates that peptides are more deeply buried in the bilayer in PC:PG membranes compared to PC bilayers. Dual Quencher Analysis (DQA) confirmed that the peptides inserted more deeply in PC:PG membranes overall, but could insert into PC bilayers at pH conditions above the pKa of the His. Circular Dichroism (CD) was used to determine the secondary structure and show that in solution the peptide appears to adopt an unstructured conformation while they become helical upon binding to the bilayer. Experiments are currently being conducted to further investigate aggregation properties in solution.

**423-Pos****Membrane Remodeling Induced by a pH Dependant Pore Forming Peptide via Atomic Force Microscopy**

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One hallmark of a cancer cell is the slightly acidic environment which is found in its immediate vicinity. It is of interest, therefore, to design mechanisms which can deliver cargo—drugs, nanoparticles, etc.—across membranes under acidic conditions only. Such delivery would bypass healthy cells in favor of cancerous ones. One method to deliver cargo is to create pores selectively under acidic conditions. pHD108 is a pore-forming peptide which was synthetically evolved to form pores large enough to pass macromolecules under acidic conditions, but not at neutral pH (*J Am Chem Soc.* 139: 937 (2017)). Recently, we applied single molecule atomic force microscope (AFM) imaging to