418-Pos Board B198
Using Enhanced Sampling Molecular Dynamics Techniques to Probe the Thermodynamics of pHLIP Peptide Insertion into a Model Lipid Bilayer
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The molecular mechanism of spontaneous polypeptide folding and insertion into a membrane, as well as its exit and unfolding, is of interest from several perspectives, including the action of antimicrobial peptides, the folding and degradation of membrane proteins, and medical applications of pH-triggered insertion peptides. A notable example is pH (Low) Insertion Peptide (pHLIP), which is a water-soluble polypeptide derived from helix C of bacteriorhodopsin that has the ability to insert into a membrane at acidic pH to form a stable transmembrane α-helix. The insertion process takes place in three stages: pHLIP is unstructured and soluble in water at neutral pH(state I), unstructured and bound to the surface of a membrane at neutral pH(state II), and inserted into the membrane as an α-helix at low pH(state III). Our hypothesis is that, there is a connection between the free energy change of binding /insertion and pKa of insertion. To test this hypothesis, we used enhanced sampling molecular dynamics (MD) techniques (steered MD and umbrella sampling) to investigate the molecular interactions of a C-terminal-truncated pHLIP variant, pHLIP-1, with a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer. The goal is to characterize the free energy of binding from state I to state II and the free energy of insertion from state II to state III (α-helical peptide insertion) and to identify key structural aspects that govern these processes. Our results agree well with the previous experiments, providing the first steps in establishing a direct relationship between the thermodynamics and pKa of pHLIP peptide insertion.

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Specific Delivery of Auristatins to Tumor Cells using pHLIP
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Localized delivery is vital for the successful development of novel and effective therapeutics for the treatment of cancer. Currently, most targeting methods rely upon expression of protein biomarkers to promote tumor targeting and killing. Despite their promises, such strategies suffer from two major drawbacks: Biomarkers are not specific to cancer cells, which can result in off-target toxicity, and cancer cells have a tendency to evolve quickly, which can lead to a loss of biomarkers and therapy resistance. However, nearly all solid tumors have a low extracellular pH, regardless of their tissue or cellular origin. Moreover, tumors' aggressiveness and metastatic potential are fostered at low extracellular pH. For these reasons, acidosis is a hallmark of tumor progression and may provide an opportunity for tumor-targeted therapy. The targeting and delivery described herein is based on the pH(Low) Insertion Peptide (pHLIP), a unique delivery peptide that can selectively target tumors in mice based solely on their acidity rather than a specific marker. Our group focuses on developing new strategies to inhibit cancer cell growth by delivering therapeutics to cells using pHLIP. We hypothesize that the localized targeting achievable with pHLIP when combined with potent therapeutics will synergize to create an efficacious treatment for cancer. In the present study, we investigate the efficacy of pHLIP to deliver the highly potent and clinically validated microtubule inhibitor monomethyl auristatin E (MMAE) to cancer cells in vitro in a pH dependent manner. We show that pHLIP and one of its variants chosen to optimize targeting, can induce a potent cytotoxic effect in cancer cells, including triple negative breast cancer cells, against which none of the current targeting strategies are effective. Furthermore, we demonstrate that these pHLIP-MMAE drug conjugates effectively target breast tumors in mice.

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The Triatoma Virus Structural Protein VP4 Induces Membrane Permeability through Dynamic Pores
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In naked viruses, membrane breaching is a key step that must be performed for genome transfer into the target cells. Despite its importance, the mechanisms behind this process remain poorly understood. The small protein VP4, codified in the genome of most Picornavirales order viruses, has been shown to be involved in membrane alterations. Here we have analyzed the permeabilization activity of the natively non-myristolated VP4 protein from Triatoma virus (TrV), a virus belonging to the Dicistroviridae family within the Picornavirales order. The VP4 protein was produced as a Maltose Binding Protein (MBP) fusion to achieve its successful expression. This recombinant VP4 protein is able to produce membrane permeabilization in model membranes in a lipid-dependent manner. The membrane-induced permeability was also influenced by the pH, being greater at higher pH values. We demonstrate that the permeabilization activity elicited by the protein occurs through discrete pores that are reversibly inserted on the membrane. Sizing experiments using fluorescent dextrans, cryo-electron microscopy imaging and other additional techniques showed that recombinant VP4 forms heterogeneous proteo-lipidic pores rather than common proteinaceous channels. These results show that VP4 protein is involved in the membrane alterations required for genome transfer or cell entry steps during dicistrovirus infection.

421-Pos Board B201
Predicting Functional Interactions in the Influenza Hemagglutinin Transmembrane Domain Via Simulation
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The precise mechanism of cell entry by influenza remains poorly understood, despite many years of research. Entry into the cytoplasm is preceded by a membrane fusion event between the virion and endosomal membrane, mediated by the fusion protein hemagglutinin. Hemagglutinin resides in the viral membrane with one transmembrane helix. Its ectodomain is trimeric, and this enforces close proximity between the three helices in the viral membrane. Previous research has demonstrated that the transmembrane domain of hemagglutinin is crucial to its pathogenicity, and that while some mutations are allowed, full viral fusion to the target membrane is blocked by drastic deletions or replacement with a GPI anchor. We have conducted molecular dynamics simulations of the hemagglutinin transmembrane domain to understand any interactions that may occur between the domains and postulate how they can impact hemagglutinin function. Preliminary results indicate that the helices can associate in a membrane, without the ectodomain present. We have used a multiscale simulation approach to examine the stability of encounter complexes in order to identify key interactions and predict changes that would disrupt them. First, coarse-grained simulations were used to generate encounter complexes between helices, which were then simulated at atomic resolution to test the stability of each complex and identify specific interactions in the membrane. Our goal is to discover stabilizing interactions that can shed light into functional data on possible hemagglutinin transmembrane domain mutants.

422-Pos Board B202
Randomly Selected Hydrophobic Peptides Inhibit Lassa Pseudovirus
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Lassa fever is a hemorrhagic fever endemic in West Africa. Currently the only available treatment, ribavirin, is only effective in the early stages of infection. Due to the limited efficacy of ribavirin, new drugs are needed to combat Lassa fever. In recent years many peptides have been developed as entry inhibitors against numerous viruses. Many of these peptides share a common trait; they are somewhat hydrophobic and have a tendency to partition into membranes. It is hypothesized that many of these peptides disrupt the physical chemistry of the membrane, inhibiting the virus from fusing with the host cell. In this study hydrophobic membrane interacting peptides were selected and challenged against a Lassa pseudovirus. This pseudovirus is composed of an HIV core (pSG3Δenv) and the Lassa envelop (Josiah strain), and allows us to study the virus, a BSL-4 pathogen, in a BSL-2 environment. Infection of the pseudovirus was detected using TZM HeLa cells, a cell line containing a gene that encodes luciferase which is promoted by tat, an HIV core protein. Initially ten hydrophobic peptides were selected; nine of which were discovered for characteristics other than antiviral activity. Interestingly, some were found to inhibit the virus while others increased viral activity. Of the peptides that inhibited the virus, the two peptides that showed the greatest antiviral activity were discovered from the same peptide library. More peptides from that library were then tested against the Lassa pseudovirus, most of which showed antiviral activity and low toxicity. In the next phase of this work these peptides will provide an archetype for the design of a combinatorial peptide library to optimize Lassa viral entry inhibition.

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Bilayer Perturbation is a Predictive Parameter for Antimalarial Development
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There is a constant need for new malaria drugs because of the perpetual development of resistance to current therapies. To facilitate the costly drug