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The 27th Annual
Harry C. Allen Jr. Symposium

Twenty-seventh Annual
Harry C. Allen Jr. Symposium

Undergraduate Student
Poster Abstracts

April 8, 2017
Poster 1

“Determining the Inhibited Structure of Trehalose-6-Phosphate Phosphatase”

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Trehalose-6-phosphate phosphatase (T6PP) is a member of the haloalkanoic acid dehalogenase superfamily (HADSF) that acts upon trehalose-6-phosphate, an intermediate in the pathway by which glucose-6-phosphate is converted to trehalose for energy production and metabolism. The T6PP gene sequence is conserved among bacterial pathogens, such as *Mycobacterium tuberculosis*. In turn, these pathogens utilize trehalose to synthesize cord factor, a mycolic acid-derived membrane glycolipid enabling toxicity, pathogenicity, and phagocyte survival. According to prior research, knockout of the otsB gene encoding T6PP in *M. tuberculosis* precludes pathogenic growth in laboratory culture and virulence in a mouse model. If a T6PP inhibitor were present, the causative agent of tuberculosis would lack the ability to cause disease.

Due to the 76% sequence identity between *M. marinum* and *M. tuberculosis* T6PP, our study investigates how T6PP crystallization from the model organism, *M. marinum*, can shed light on the structure of the *M. tuberculosis* protein. In order to minimize aggregation and increase homogeneity, *M. marinum* T6PP was purified via sonication and fast protein liquid chromatography (FPLC), a screen was conducted to ascertain which buffer would maximize yield, and the protein was analyzed via dynamic light scattering (DLS). In addition, a grid screen was designed to determine the solution optimal for protein crystallization and microcrystals were produced via sitting drop vapor diffusion. In the future, synchrotron radiation and SAXS (small angle X-ray scattering) will be employed in order to determine the three-dimensional structure of *M. tuberculosis* T6PP in the presence of substrate and competitive inhibition.

Poster 6

“Patient-derived melanoma variants of DNA polymerase theta exhibit altered polymerase activity”

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DNA polymerases play an important role in maintaining the genome during DNA repair, but poorly functioning DNA polymerases can contribute to genetic instability through misincorporation of base pairs. DNA polymerase Theta (Pol θ, POLQ) performs low fidelity repair of DNA damage, is upregulated in breast cancer, and associated with poor survival rates. We have identified POLQ mutations from patient-derived melanomas and hypothesize that these variants will have altered polymerase activity compared to wild-type. Variants L2538R and E2406K, located in the palm domain and fingers domain, were generated via site-directed mutagenesis and expressed/purified from *E.coli*. The variants were assayed for qualitative DNA polymerase activity and preliminary studies suggest that the variants demonstrate a propensity to misincorporate nucleotides resulting in altered base-selection activity compared to wild-type. This suggests melanoma derived Pol θ mutations repair DNA differently compared to wild-type, which may contribute to overall genomic instability.
Poster 5

“Sorption of Pyrene by Commercially Available Water Filters”

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Water filters are widely used to reduce or remove sediment, chlorine taste, and odor from drinking water. In addition to these applications, some water filters are designed to remove heavy metals, organic pollutants, and some parasites. One class of potentially carcinogenic pollutants that can be found in water are polycyclic aromatic hydrocarbons (PAHs) which are produced from the incomplete combustion of fossil fuels. In this study, batch type sorption experiments were used to analyze the interaction between pyrene (a common PAH) and the material used in two commercially available water filtration systems.

Poster 2

“Investigation of Human Pol Eta’s Role in Translesion Synthesis Past Cisplatin-Induced DNA Damage”

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DNA replication is performed by a group of enzymes called DNA polymerases. A special class of DNA polymerases, called the Y-family polymerases is able to traverse DNA lesions. Cisplatin is a chemotherapeutic agent that damages DNA and has been used to treat cancer. However, many patients become resistant to cisplatin. The potential role of Y-family DNA polymerases in the pathway of chemotherapeutic resistance has been under intense investigation. Growth, purification and expression of the Y-family DNA polymerase, Pol eta (Pol\(\eta\)) was accomplished by recombinant methods and FPLC (Fast Protein Liquid Chromatography). SDS polyacrylamide gel electrophoresis was used to visualize the success of the purification, and Bradford assay was conducted to quantify the amount of protein purified. A strand-displacement assay as well as primer-template assay was used to verify the protein's ability to synthesize synthetic DNA. The long-term goal of this research is to determine the molecular details by which Pol\(\eta\) is able to bypass DNA damaged by cisplatin. Furthermore, this work provides the foundation for future experiments that include screening chemical libraries that could potential inhibit Pol\(\eta\).
**Poster 3**

“Helix-dipole effects on the formation of amyloid fibrils in the presence of membranes”

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Islet amyloid polypeptide (IAPP) is a hormone cosecreted with insulin by pancreatic β cells. Under certain conditions, IAPP aggregates to form amyloid fibrils that are toxic to cells. Understanding the process of fibril formation is therefore of great medical relevance. Recently, Liu et al. (Biochemistry 2012, 51. 4167-4174) showed that helix-dipole effects modulate fibril formation by amyloidogenic fragments of IAPP in water. Here, we studied the aggregation of IAPP(11-25)S20G (R_{11}LANFLVHSGNNFGA_{25}-NH$_2$), one of the most amyloidogenic fragments of IAPP, in the presence of model membranes. Using circular dichroism, ThT fluorescence and electron microscopy, we show that IAPP(11-25)S20G does not aggregate in the presence of neutral model membranes. In the presence of anionic and partially anionic membranes, however, α-helices were formed and fibril formation occurred. These results strengthen the hypotheses that the α-helix is on the pathway to amyloid formation and that the helix dipole is an attractive target for the identification of modulators of fibril formation.

**Poster 4**

“HECTD1 – An E3 Ubiquitin ligase Involved in Neural Tube Closure and Placental Development”

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Homologous to E6AP C-Terminal Domain 1 (HECTD1) is an E3 Ubiquitin Ligase involved in the ubiquitin signaling pathway. HECTD1 has been expressed in the labyrinth zone of the placenta where the exchange of nutrients and waste occurs between maternal and fetal circulatory systems. Genetic mutations in HECTD1 result in abnormalities in the structure of the labyrinth zone. Furthermore, incomplete penetrance of HECTD has shown to be responsible for neural tube defects, such as exencephaly and spinabifida. Our research goals are to biochemically characterize the C-Lobe of the HECT Domain of HECTD1 to ultimately prevent associated diseases. Thus far, our efforts have demonstrated a melting point of the C-Lobe to be 45.4°C, with a largely alpha helical structure. To further understand the importance of the conserved NTC residues, associated mutations of these residues were created.