UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2019 Mary Gates Hall

# Online Proceedings

## SESSION 1Q

### BIOLOGICAL STRUCTURE AND FUNCTION

*Session Moderator: Matt Kaeberlein, Pathology*
**JHN 022**
*12:30 PM to 2:15 PM*
* Note: Titles in order of presentation.

**Elucidatidating the Binding Interaction of the *LINK-A* lncRNA to $PIP_3$ Using Nuclear Magnetic Resonance (NMR) Spectroscopy**
*Angelique Amado, Recent Graduate, Chemistry, University of Washington*
*Howard Hughes Scholar, UW Post-Baccalaureate Research Education Program*
*Mentor: Gabriele Varani, Chemistry*

Long non-coding (lnc)RNAs have multiple biological functions, including recruitment of kinases to regulate signaling pathways involved in tumorigenesis and other human diseases. Of particular interest is the proposed interaction between *L*ong *I*ntergenic *N*oncoding *RNA* for *K*inase *A*ctivation (LINK-A) and the membrane component phosphatidylinositol-3,4,5-triphosphate ($PIP_3$). The proposed interaction between $PIP_3$-LINK-A would be the first example of a direct interaction between a non-coding RNA and phospholipid. I investigated the proposed interaction between LINK-A and $PIP_3$ using Nuclear Magnetic Resonance (NMR) spectroscopy. Multiple NMR-based experiments were performed to assess the degree of binding on the basis of line-width broadening of NMR spectra. Wild-type and mutated RNA constructs were titrated into a 100 uM solution of $PIP_3$, but the NMR data showed no evidence of line-width broadening, indicating that no direct interaction occurs between wild-type or mutated RNA constructs. Presumably, the reported cellular interaction is not direct and might require an additional mediating factor. I determined the 3D structure of the LINK-A RNA hairpin, required to recruit $PIP_3$, using biophysical molecular modeling and NMR data. My results provide a biophysical foundation to elucidate the functional role of LINK-A in $PIP_3$ recruitment and kinase activation.

## POSTER SESSION 2

**Balcony, Easel 93**
*1:00 PM to 2:30 PM*

**Preparation of a Promoter Associated Non-coding RNA CDH1 -160(A) Using a Downstream HDV Ribozyme Sequence to Produce Homogenous Transcripts and Improve NMR Visibility**
*Lauren Yvette Marie Cominsky, Junior, Biochemistry*
*Mentor: Gabriele Varani, Chemistry*
*Mentor: Matt Walker*

Long non-coding RNAs (ncRNAs) play a significant role in transcriptional regulation; therefore, mutations in their sequences can lead to human disease. An example is provided by the promoter associated non-coding RNA (paRNA) that, when bound with Argonaut 1 (Ago1) and a miRNA, plays a key role in coordinating gene silencing of the tumor suppressor CDH1 in epithelial cells. The paRNA expression can be implicated with cancer. This is due to a single nucleotide polymorphism (SNP) at positions -160(C/A) relative to the CDH1 promoter. The -160(A) isoform favors a unique secondary structure of the paRNA, distinct from more common -160(C), which favors over-suppression of CDH1 and leads to increased cancer risk. To better understand how the -160(A) isoform mechanistically drives increased suppression of CDH1, our lab is applying NMR-based methods to determine the 3D structure of the paRNA. However, the T7 RNA polymerase used to synthesize RNA is prone to producing heterogeneous products in longer RNAs such as this one. Having exact lengths of the transcript is important for improving signal-to-noise and peak sharpness in NMR spectra, which is critical for 3D structure determination. To overcome this problem, I designed a pUC19 plasmid containing the paRNA -160(A) sequence with a downstream self-cleaving hepatitis delta virus (HDV) ribozyme. Following transcription, the HDV ribozyme undergoes self-cleavage, producing homogenous ends at the 3' end of the paRNA. My results show that HDV incorporation produces a single species of RNA with no 3' overhang and improves NMR spectra quality. This cloning tool is easily adaptable to other large RNAs, facilitating data collection for other large RNAs used in our lab. Ultimately, our ability to predict the 3D structure of this paRNA could someday lead to its use as a potential drug target.