UNIVERSITY OF LOUISVILLE

COLLEGE OF ARTS & SCIENCES

Department of Chemistry

Graduate Student Research Seminar
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CBLL16

Liquid Chromatography-Mass Spectrometry Based Epitranscriptomics

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RNAs are biomolecules in all life that have a significant impact on transmitting genetic information, catalyzing biological reactions, and participating in protein synthesis 1, 2. RNAs are produced from information encoded in DNAs by transcription. The collection of all RNAs in a cell is referred to as the transcriptome. The exciting discovery that mRNA modifications can regulate protein production paved the way for the emerging field of epitranscriptomics, which seeks to elucidate the role of RNA modifications in regulating gene expression at transcriptome level. RNA therapy, treating and preventing diseases using RNA-based molecules, has become a major research area, especially in response to the COVID-19 pandemic1. In my research seminar, I will present my work on liquid chromatography-mass spectrometry (LC-MS) based epitranscriptomics. Nanopore direct-RNA sequencing (DRS) is a widely used technology to simultaneously quantify multiple RNA modifications with single base resolution. However, DRS has low sensitivity and cannot detect novel modifications 2. I developed an innovative Zn-mediated RNA hydrolysis method to cleave RNA into oligonucleotides (OGNs) and further analyze the OGNs by reversed phase liquid chromatography-mass spectrometry (RPC-MS) to identify and quantify OGNs. My study shows that the Zn-mediated cleavage is random, resulting in not only longer OGNs but also multiple OGNs with sequence overlap. Compared with RNases T1 and A digestions, the Zn-mediated cleavage provides excellent sequence coverage, i.e., 70% and 83% of 28S rRNA in HepaRG cells and E. coli, respectively, and more than 60% and 79% of 18S rRNA. Furthermore, identification of the same chemical modification in different OGNs with sequence overlap greatly increases the confidence of assigning the modified OGNs to RNA sequences. These findings show that the Zn-mediated cleavage can be an alternative to the standard enzymatic cleavage procedures, allowing for more extensive RNA analysis and advancement of RNA sequencing for epitranscritpomics.

References:

1. Alfonzo, J. D., Brown, J. A., Byers, P. H., Cheung, V. G., Maraia, R. J., & Ross, R. L. Nature genetics, 2021, 53(8), 1113-1116.
2. Vanhinsbergh, C. J., Criscuolo, A., Sutton, J. N., Murphy, K., Williamson, A. J., Cook, K., & Dickman, M. J. Analytical Chemistry, 2022, 94(20), 7339-7349.
3. Jebet, A., Xu, R., Fu, X.; He, L., Mueller, G. E., Zhang, X. manuscript in preparation.