

Analytical Chemistry Seminar

Tuesday, November 14, 2023
3:30 p.m. ~ WTHR 320

"Protein Measurement via Single-Cell Immunoblotting (scIB) coupled to Multiplexed Ion Beam Imaging - Time of Flight (MIBI-TOF)"



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Abstract:

Single-cell measurements are key to developing biological understanding of heterogeneous cell populations. Currently, RNAseq is the method of choice for single-cell measurements, but mRNA abundance is poorly correlated with protein abundance and provides no insight in protein modification state. Immunoblotting (IB) techniques are useful for identifying the abundance, size, or modifications of a target protein within the sample. However, these methods require a large amount of protein, and the result is typically imaged via fluorescent techniques, which limits multiplexing abilities due to the spectral overlap of multiple fluorophores. Dr. Amy Herr at University of California – Berkley developed a single-cell western blotting (scWB) technique, which can also be applied to isoelectric focusing (IEF), in order to separate the proteome of a single cell. To solve the multiplexing problem, metal-tagged primary antibodies were introduced to label the proteins rather than the untagged primary antibody and fluorophore-tagged secondary antibody standard; these samples are analyzed via multiplexed ion beam imaging – time of flight (MIBI-TOF). It has been demonstrated that scIB-MIBI-TOF is able to achieve proteoform-resolved measurements, but this is limited by the number of commercially available antibodies available. scWB-MIBI-TOF is a novel method that has the potential to provide insight into the diverse and complex proteome of a single cell.

Teagan Campbell is a second-year graduate student. She graduated from King's College in 2022 with her B.S. in chemistry. While there she participated in organic research under Dr. Jillian McCue where she worked on synthesizing water-soluble porphyrins to detect and stabilize quadruplex DNA. The overall goal of this research was to inhibit telomerase activity in cancer cells; telomerases extend the telomeres of chromosomes which prevents cell death. Last fall Teagan joined Dr. Bryon Drown's lab where she studies the proteoforms of kinases as well as their interactions with small-molecules through chemical top-down proteomics.