MSc DEFENCE Thursday, September 26th, 2019
Student: Samuel Weng
**Title: DEVELOPMENT OF HIGH-EFFICIENCY UNDECANAL-BASED N TERMINI ENRICHMENT (HUNTER)
FOR MONITORING PROTEOLYTIC PROCESSING IN LIMITED SAMPLES**
Time and location: 10:00 AM; Room 3113, BC Children's Hospital Research Institute, 938 West 28th
Avenue, Vancouver, BC
Supervisor: Dr. Philipp Lange

ABSTRACT

Protein N termini can identify truncated, alternatively translated, or modified proteoforms with distinct
functions. Following the occurrence and loss of protein N termini can also serve as indicators of
abnormal proteolytic activity driven by deregulated proteases and reveal perturbation in defects and
cancers.

Selective enrichment of N terminal peptides is necessary for proteome-wide coverage for unbiased
identification of site-specific proteolytic processing and protease substrates. However, for
comprehensive N terminome analysis, most N termini enrichment techniques require relatively large
amounts of starting material; the typical range is several hundred micrograms to milligrams of crude
protein lysate. Due to sample constraints, this type of analysis is unfeasible for clinical biopsies,
especially those from pediatric patients.

We present High-efficiency Undecanal-based N Termini EnRichment (HUNTER), a robust, sensitive, and
scalable method for the analysis of previously inaccessible microscale samples. With this approach,
>1,000 N termini are identified from a minimum of 2μg raw HeLa cell lysate and >5,000 termini from
200μg of raw HeLa lysate with high-pH pre-fractionation. We demonstrate the broad applicability of
HUNTER with the first N terminome analysis of sorted human primary immune cells and enriched
mitochondrial fractions from pediatric cancer patients. The workflow is further implemented on a liquid
handling system to demonstrate the feasibility of clinical degradomics by automated processing of liquid
biopsies from pediatric cancer patients as well as the benefits in handling rare and precious clinical
samples.