A Fit-for-Purpose Approach to Detect Amino Acid Substitutions in Shotgun Proteomics

Matthew Champion

Ph.D., Associate Professor, Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN, USA

The Champion group is primarily interested in developing and exploiting novel approaches to identify and characterize the components of secreted proteins from virulent microorganisms. We heavily utilize the 'awesome power of genetics' coupled with state-of-the art quantitative proteomics to enrich, identify and quantify the proteins responsible for biological phenotypes. We have ongoing projects in pathogenic mycobacteria, protein translation in E. coli through the PTRN, and quantitative protein secretion measured using capillary electrophoresis.

We generated a defined positive and negative library containing 82,843 spectra representing 4,161 unique peptide sequences by mixing E. coli and S. typhimurium. In-silico digestion resulted in a comprehensive list of 31,053 potential AAS peptides and included cleavage rules to generate predicted peptides arising from scissor substitutions. Scissor substitutions are cases where a substituted peptide loses or gains a protease cleavage site. An example of this would be X->K/R which introduces a new tryptic cleavage. A priori, scissor substitutions are ambiguous in enzymatic search algorithms that apply protease logic prior to spectral-mass matching. We found reasonable success applying MSFragger’s mass-offset search to identify AAS target S. typhimurium peptides using only an E. coli search database. Overall, 62% of target sequences were identified. Target identification decreased in serial dilution for both mass-offset and concatenated database search. This implies that identification of substituted peptides is limited by conditions that reduce identification of all peptides. The establishment of the target AAS library thus enabled systematic evaluation of individual components toward identification of substituted peptides. We demonstrate that this fit-for-purpose mixed-proteome can direct improvements in acquisition and shotgun search strategies for detection of substituted peptides.