# Proteomic Techniques in the Physiological Proteomics Core Facility

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## Abstract

A new software package, IdentiQuantXL, has been developed in the Physiological Proteomics Core Facility to provide large-scale protein identification and label-free quantification using either low or high resolution LC-MS/MS data.

Though many software packages have been developed to perform label-free quantification of proteins in complex biological samples using peptide intensities generated by liquid chromatography - tandem mass spectrometry (LC-MS/MS), two important issues hinder the use of peptide intensity measurements: (i) It is difficult to accurately determine the retention time of each peptide peak, especially for low resolution data, and (ii) many peptides cannot be used for protein quantification. To address these two key issues, we have developed a new method to enable accurate peptide peak retention time determination and multiple filters to eliminate unqualified peptides for protein quantification. Repeatability and linearity have been tested using ion trap-derived low resolution data from six very different samples, i.e., standard peptides, kidney tissue lysates, HT29-MTX cell lysates, depleted human serum, human serum albumin-bound proteins, and standard proteins spiked in kidney tissue lysates. In all these unique experiments, at least 90.8% of proteins (up to 1,390) had CVs ≤ 20% across 10 technique replicates, and at least 92.1% of proteins (up to 2,013) had $R^2 \geq 0.9500$ across 7 concentrations. The performance of our strategy was verified using identical amounts of standard protein (lysozyme) spiked in complex biological samples (cell culture media containing secreted proteins) with a CV of 8.6% across eight injections. The excellent performance was further confirmed by comparing label-free mass spectrometry to Western blot detection of prolactin, which was decreased 17.1-fold in dwarfed mice compared to wild-type using the label-free quantification strategy and very low or undetectable using Western blot. The results indicate that our new platform, named IdentiQuantXL, accurately quantifies thousands of peptides and proteins in complex samples. It has been applied in the aqueous humor proteome in patients with Fuchs endothelial corneal dystrophy. While many software packages focus only on high resolution data, our strategy is designed for both high and low resolution data. Consequently, it is very useful for data generated by low resolution mass spectrometers such as the LTQ, especially when the dynamic exclusion of ions in data acquisition is enabled to obtain more MS/MS fragments of low-abundance peptides to maximally identify proteins in a complex biological sample. Supported by NIEHS RC2ES018810 and NIGMS R01GM085218