

Bankhead-Coley Cancer Research Program

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Project Title: Acquisition of Hybrid Quadrupole Ion Trap Mass Spectrometer for Proteomics

Project Summary: This grant for cancer research at the H. Lee Moffitt Cancer Center and Research Institute includes a liquid chromatography coupled to mass spectrometry (LC-MS) system featuring a hybrid triple quadrupole ion trap mass spectrometer (4000 QTrap, Applied Biosystems). This mass spectrometer combines the functions of triple quadrupole mass spectrometers, which set the standard for monitoring specific molecules in complex mixtures, and ion trap instruments, which are powerful tandem mass spectrometry (MS/MS) tools for peptide sequence analysis. Thus, the QTrap is used for specific molecular monitoring and targeting, yet still provides peptide sequence verification using MS/MS. As such, this LC-MS system can validate candidate biomarkers for diagnosis, prognosis, and chemoprediction; it can also selectively identify the events that form the molecular basis of cancer by targeting peptides with particular types of post-translational modifications based on their fragmentation signatures. The QTrap LC-MS system instrument not only complements existing instrumentation, but also enriches analyses for relevant biological phenomena. To validate candidate biomarkers, the QTrap LC-MS system is used to specifically quantify carefully selected individual peptides from complex mixtures (e.g., proteolytic digests of blood plasma or urine) using a technique known as multiple reaction monitoring (MRM). Proteolytic peptides from each candidate protein biomarker are analyzed with LC-MS/MS to measure their elution time, the mass to charge ratio (m/z) of the intact peptide, and the fragment ions produced during MS/MS. Using these three characteristics to restrict the analysis, the QTrap can be programmed to quantify several target molecules in a single run by recording the intensity of a selected fragment ion from each target peptide. MRM enables relative quantification of the peptide and its protein of origin by comparison of this fragment ion signal across different samples. Absolute quantification can be performed using standard curves or the incorporation of known amounts of heavy isotope-labeled standards. This method can be used for rapid analysis of a large population of samples, validating the biomarker without the time lag involved in antibody development. Because of its unique design, the QTrap can perform neutral loss and precursor ion scans, enabling the selection of peptide precursors containing specific types of post-translational based on their gas phase fragmentation patterns. For example, the QTrap can selectively detect, fragment, sequence, and identify phosphopeptides using precursor ion scans observing negative fragment ions at m/z 79, which correspond to HPO_3^- . The instrument scans through the entire m/z range of peptide precursors, fragments the phosphopeptides, and reports negative fragment ion signal intensity at m/z 79. Once a peak is observed, the peptide precursor is selected and fragmented in the mass spectrometer. MS/MS is only triggered by negative ion signal at m/z 79; therefore, the mass spectrometer is able to target an important biological process, phosphorylation, at the molecular level. This technique affords detection of low levels of post-translational modification in digests of individual proteins; it can also be used for proteome scanning experiments, which target the discovery of novel phosphorylated sequences. In such an experiment, previously

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unknown phosphopeptides can be detected in complex mixtures (e.g., digests of protein complexes), enabling the analysis of signaling pathways, distinguishing relevant downstream events, and possibly defined novel drug targets. Additional experiments will be designed around the fragmentation patterns of other post-translational modifications. As described in the two examples above, the QTrap system will help establish candidate biomarkers, elucidate the molecular mechanisms involved with cancer, and play a vital role in establishing a molecular basis for diagnosis and treatment as well as identifying novel molecular targets for therapy.