2019 ASMS Conference Abstract

Title: Automated PTMscan® immunoaffinity enrichment for the capture of KGG modified peptides from complex mixtures

Authors: Lilian Phu, Shadie Nimri, Anne Baldwin, Nadia Martinez Martin, Chris Suh, Matthew P. Stokes, and Donald S. Kirkpatrick

Introduction (120 words):
Recent advances in mass spectrometry instrumentation and sample handling have enabled researchers to routinely perform global profiling of many protein post-translational modifications, expanding our knowledge of biological pathways. Key to the success of these experiments is the effective selective enrichment of the modified peptides from complex mixtures before introduction to the mass spectrometer, often via immunoaffinity purification using antibodies that are directed against the PTM of interest. Here, using the ubiquitin remnant motif (KGG) antibody as a model, we expand on the PTMscan® immunoaffinity enrichment protocol by coupling it to the Phynexus MEA robot, developing a robust automated platform that enables the concurrent processing of up to twelve samples with limited manual sample handling. We demonstrate the utility of the automated system in the identification of thousands of KGG peptides from complex biological samples.

Methods (120 words):
Parameters including duration of peptide incubation, temperature, washing steps, resin type, and covalent antibody-resin coupling have been investigated for their effects on enrichment efficiency. Optimization experiments were performed on both a simple KGG peptide mixture and peptides derived from complex cell lysates. Qualitative and quantitative performance of the automated system was evaluated via MALDI-TOF MS and LC-MS/MS.

Preliminary data (300 words):
Temperature studies revealed enrichment performed at 4^{o}C increased the efficiency of peptide capture compared to room temperature. In experiments investigating the performance of various protein-A resin formats, optimization improved KGG peptide recovery from 6-25%, while minimizing nonspecific peptide binding. Subsequent experiments on the automated system exploring covalently coupled antibody showed a similar number of KGG peptide identifications as compared to enrichment using the non-crosslinked antibody. However, a three-fold increase in nonspecific peptides was observed with crosslinking, which has the potential to negatively impact KGG-peptide identification rates. Based on this data, non-crosslinked antibody was selected for the automated protocol. The optimized automated immunoaffinity enrichment protocol increased throughput ~5 fold without additional manual sample preparation time. Automation has proven valuable in multiplexed quantitative experiments where >10 samples are typically simultaneously processed.

Novel Aspect (20 words):
Development of an automated platform for performing PTMscan® immunoaffinity enrichment of KGG peptides

Figure1. Effects of temperature on peptide capture

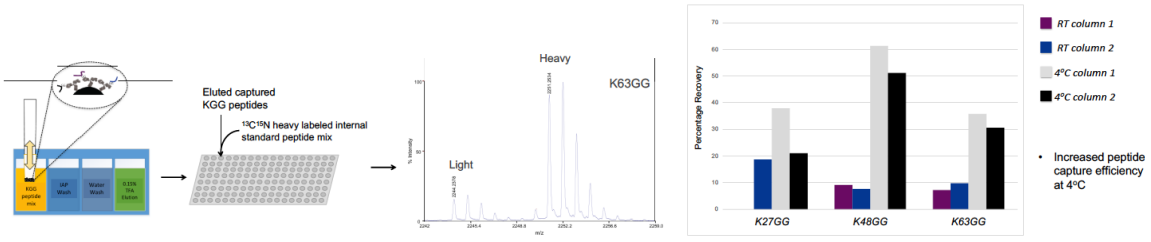

Figure 2. Automated and Manual IAP are comparable

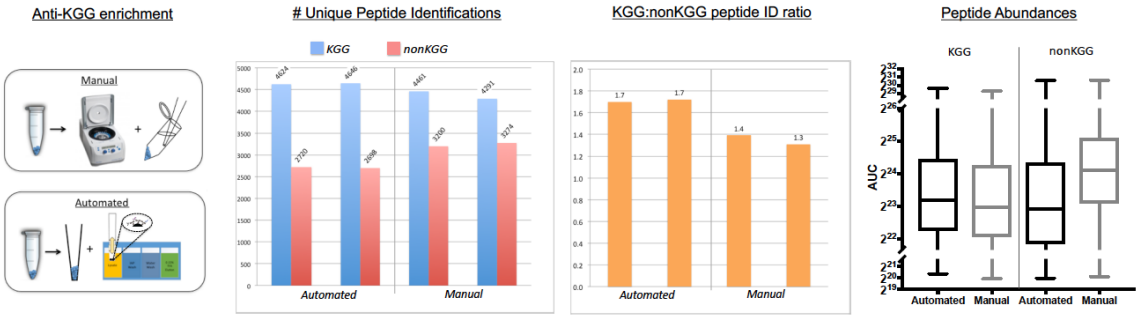

Figure 3. Evaluation of Protein A resins for automated method

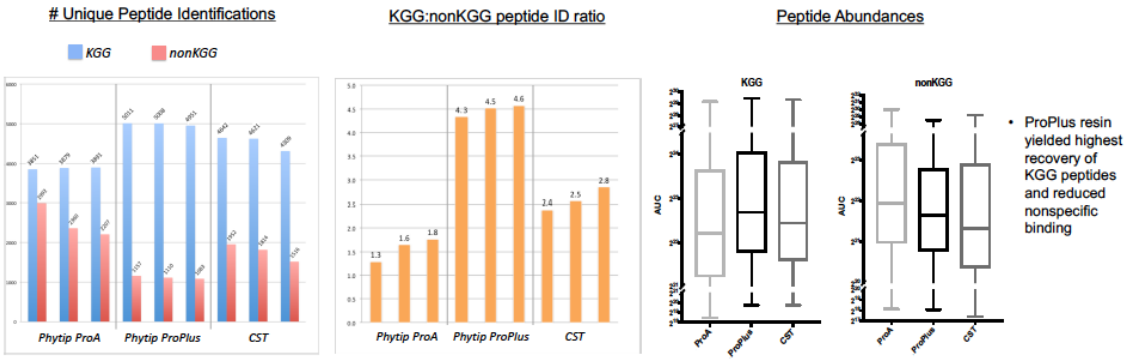

Figure 4. KGG automation matches manual performance with increased throughput and efficiency

	MANUAL	AUTOMATED
MANUAL HANDLING TIME	~1 to1.5 hr	~15 min
REPRODUCIBILITY	Variability (individual tubes handled sequentially)	Uniformity (all columns processed concurrently)
CAPACITY	8-10 per 1.5 hr hands-on time	60 per 1.5 hr hands-on time