

## **Quantifying peptide phosphorylation using MALDI-TOF mass spectrometry**

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**ABSTRACT:** Protein phosphorylation by kinase enzymes is one of the most common signals used by intracellular communication pathways. The regulation of cellular processes such as growth and proliferation by these phosphorylation events is extremely important for the overall normal function of the cell. Disruption in the levels of phosphorylation in any given pathway can result in uncontrolled behavior, leading to diseases such as cancer. Traditional methods for monitoring protein phosphorylation are limited by their reliance on specific antibodies for detecting particular phosphorylated proteins. Mass spectrometry allows an unbiased, label-free approach to detecting even unexpected phosphorylations. However, differences in ion chemistry between unphosphorylated and phosphorylated peptides present a challenge for performing quantitative analysis of the level of phosphorylation using mass spectrometry. Therefore we designed a series of experiments to 1) calibrate the MALDI-TOF mass spectrometric behavior of peptide/phosphopeptide pairs and 2) investigate the contributions of chemical and mechanical parameters of the sample preparation and the instrument on the relative ionization/detection of these peptide pairs. We found the behavior to be reproducible but not easily predictable by peptide sequence. We are currently developing informatic tools to further analyze and understand the ionization behaviors and eventually develop predictive models for other peptide/phosphopeptide pairs.

**ACKNOWLEDGEMENT:** We thank David Jabon (DePaul University) for input on data processing and representation. Aaron Engel-Hall and George Steinhardt were supported by the REU Summer Undergraduate Research program at DePaul University. Laurie Parker is supported by the NIH through an NRSA Individual Postdoctoral Fellowship.

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