



Metabolic Profiling of Phosphorylated and Coenzyme-Bound Metabolites Using Pressure-Assisted Capillary Electrophoresis Mass Spectrometry



- **Purpose:**

- Metabolic profiling of phosphorylated carbon intermediates of the respiratory pathways, adenylates, nucleotides, and other highly charged metabolites, such as coenzyme-bound metabolites, is critically important, given their involvement in redox balance and energy transduction.
- Such highly-charged metabolites are not being adequately addressed in Genomes-to-Life studies that require metabolomic analyses to establish the health and metabolic status of microbial cultures and the metabolic consequences of genetic manipulation.
- Instrumentation is now available to study these metabolites simultaneously, with much higher throughput, and in conjunction with metabolites of interdependent pathways.
- We propose to assemble and test a hybrid instrument to separate and quantify low concentrations of these metabolites by interfacing a soon-to-be acquired pressure-assisted capillary electrophoresis (PACE) system in tandem with ion trap and/or quadrupole time-of flight mass spectrometers (MS).
 - ▶ When coupled with stable isotope-labeled precursors that are tracked in time-series sampling, it will provide an assessment of flux for these metabolites. Data extraction and deconvolution strategies to separate co-eluting metabolites will be constructed for PACE- MS that address both static and dynamic analyses.
- Research activities will include the assembly of a database of metabolites that can be profiled by PACE-MS, identifying key mass fragments and the retention time of each metabolite, and test analyses of time-series tracking of ¹³C-glucose catabolism by microbial cultures.

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