Development of Robust, Reproducible, High-Throughput Proteomic Assays for Cellulosic Biofuel Applications

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Project Goals:

Over the past 10 years, the bioenergy field has realized significant achievements centered on biosynthetic production of fuel-like compounds. Key to the success of these efforts has been transformational developments in metabolic engineering of biofuel-producing microbes. To aid these efforts, we have developed proteomic methods based on standard flow UHPLC-MS to characterize and quantify complex samples. By using standard flow UHPLC-MS, we were able to routinely identify nearly 800 proteins from *E. coli* samples; while for samples from *Arabidopsis thaliana*, over 1,000 proteins could be reliably identified[1]. To increase sample throughput, we developed automated sample preparation protocols and fast chromatography methods for quantitative targeted proteomic experiments[2]. We shortened the time and minimized the effort to target new proteins by implementing retention time prediction method that allow direct transfer from shotgun proteomics data to short gradient targeted proteomics methods. We demonstrated this workflow on a variety of *E. coli* single gene knockout mutants and other hosts relevant to bioenergy applications.

References

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