Title: An semi-automated workflow for high-throughput and quantitative proteomic analysis of metabolic engineered microorganisms

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Project Goals:

JBEI's mission is to establish the scientific knowledge and new technologies in feedstock development, deconstruction and separation, and conversion needed to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts.

Abstract:

Production of advanced biofuels and valuable chemicals through microbial fermentation contributes to more sustainable energy and chemical resources. Mass spectrometry (MS)–based proteomic analysis plays an important role in understanding the function of engineered microbes to aid metabolic pathway optimization through multiple DBTL cycles. As a result, there is increasing interest in large amounts of high quality proteomic data to create new and meaningful biological knowledge. Our previous efforts have greatly advanced omics sample throughput and overall data quality by establishing a rapid targeted proteomic workflow[1] and a complete automated proteomic sample preparation platform[2], yet challenges remain in automation robustness and increasing analytical sample throughput. To tackle these challenges, we established a semi-automated workflow that combines robust modular automated sample preparation protocols and fast data independent acquisition to enable high throughput and quantitative proteomics analysis of metabolically engineered microorganisms. This strategy decreased the complexity of an existing fully-automated workflow[2], reduced variance in the sample preparation process, and circumvent bottlenecks related to automation system availability. The modular automated workflow also reduced the total sample preparation time by over 50% and simplified methods development for new organism compared to the full automated sample preparation platform. The parameters in these modular automated methods are easily adjustable so that they could be transferred to labs with standard automated liquid handlers. Concurrently, we developed high-throughput shotgun proteomic data acquisition methods that achieve routine detection and quantification of over 800 proteins within 15 minutes per sample analytical time, thus providing throughput of ~100 samples/day. We are also building deep proteome libraries of bioenergy relevant organisms containing over 1,000 native proteins and ever increasing number of heterogenous pathway proteins. Preliminary testing of this integrated workflow yields quantification of >15,000 peptides from over 1,000 proteins. From these results, over 65% of these peptides achieved a CV% below 20%, and the overall median CV% is less than 15%. In the future, we will be using this semi-automated proteomic platform to comprehensively investigate the proteome changes in engineered microbes (e.g., *Pseudomonas putida*, *Rhodosporidium toruloides*).

References

- Chen Y, et al. (2019) A rapid methods development workflow for high-throughput quantitative proteomic applications. PLOS ONE 14(2): e0211582. https://doi.org/10.1371/journal.pone.0211582
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