Characterizing the *Saccharomyces cerevisiae* glycolytic oscillation exometabolome by ion mobility-mass spectrometry

Jeffrey R. Enders,[1,3] Christina C. Marasco,[2,3] Kevin T. Seale,[2,3]
John P. Wikswo,[2,3,4] and John A. McLean[1,3]

***Short Abstract* — In this work glycolytic oscillations in yeast cells are used as a model system to explore the use of microfluidic cell trapping combined with nanoelectrospray ionization-ion mobility-mass spectrometry (nESI-IM-MS) for interpreting cellular metabolism through examination of the exometabolome. By sampling across full yeast oscillation cycles, various species of interest are singled out and investigated to determine their secreted production in oscillations.**

***Keywords* — ion mobility-mass spectrometry, Saccharomyces cerevisiae, microfluidics, nanophysiometry**

I. Purpose

Monitoring cellular responses is a difficult task due to the inherent complexity of biological samples. This is especially true when dealing with the cellular exometabolome, or the suite of biomolecules belonging to a cell's metabolome that are secreted for the purpose of cell-to-cell communication. Currently work is underway to implement novel cellular exometabolome response protocols through the use of microfluidics combined with ion mobility-mass spectrometry (IM-MS). With the aid of IM-MS, simultaneous sampling of all secreted metabolites and therefore a more complete understanding of underlying cellular mechanisms are potentially within reach. By using a microfluidic multi-trap nanophysiometer [1] to capture cells, one can direct medium over these cells and feed the effluent into an IM-MS instrument to analyze the secreted biomolecules. IM-MS is uniquely suited for determining contributions from individual biomolecular classes (*i.e.*, carbohydrates, nucleotides, proteins, lipids, and metabolites) which may be present in the exometabolome irrespective of isobaric, or nominally same mass, interferences. By these means one can quantify cellular response based on a qualitative biomolecular exometabolome profile of small numbers of cells.

Acknowledgements: This work was funded by NIH grant U01AI061223 and DTRA grant HDTRAI-09-1-001.

[1]Department of Chemistry, Vanderbilt University, Nashville, TN 37235
[2]Department of Biomedical Engineering, Vanderbilt University
[3]Vanderbilt Institute for Integrative Biosystems Research
[4]Departments of Physics & Astronomy and Molecular Physiology & Biophysics, Vanderbilt University

II. Summary of results

Preliminary work has been dedicated to streamlining the compatibility of microfluidics and nESI-IM-MS and verifying feasibility of the system with standardized peptides and proteins. Further work demonstrated the ability of the two technologies to generate time-correspondent data from live cellular systems through offline and online near-real time analysis [2]. This work was made possible by the use of online solid phase extraction and online microdialysis.

Present studies have been aimed at delineating biomolecular differences in effluent from *Saccharomyces cerevisiae* during various stages of glycolytic oscillation. Approximately 20 samples were collected from a large yeast bioreactor over the course of 8 hours. These samples were desalted prior to analysis to avoid any suppression effects the salts may have on analytes of interest. Immediate benefits to this platform were observed, such as the ability to separate out and therefore negate interferences from various polymeric medium components present in the effluent samples. Once we demonstrate the IM-MS yeast oscillation signature, we will proceed to determine whether oscillations can be maintained in the microfluidic devices alone.

III. Conclusion

Integrative technology such as that outlined here is crucial to advancing prediction and control of cellular regulatory systems. The combination of microfluidics and IM-MS allows for simultaneous measurement of all secreted biomolecules despite interferences or sample complexity. From the perspective of systems biology, the future utility of such a measurement strategy lies in small cell numbers, the rapid testing/validation of models, the possibility of using machine learning for automated model specification, and eventually the external control of cellular biosystems.

References

[1] Faley S, et al. (2008) Microfluidic platform for real-time signaling analysis of multiple single T cells in parallel. *Lab on a Chip* **8**, 1700-1712.

[2] Enders J, et al. (2010) Towards monitoring real-time cellular response using an integrated microfluidics-MALDI/nESI-ion mobility-mass spectrometry platform. *IET Systems Biology*, in press.