Monitoring of real-time protein concentrations in cellular yeast secretomes

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***Short Abstract* — The budding yeast *Saccharomyces cerevisiae* serves as an ideal demonstration system because it is well modeled, complex, and responsive. Coupling microfluidic multitrap nanophysiometer (MTNP)—to visually observe yeast within the growth phase—with automated MALDI MS plate spotting of cellular effluent output from the device provides temporal resolution of the secretome mass spectrum. By selecting specific peaks of interest and using imaging software, a map of protein concentration variance over time can be developed. Once the landscape of the cell environment is generated, we hope to manipulate multiple-variable inputs in an aim to control a predictive cell system response.**

***Keywords* — Saccharomyces, matrix-assisted laser desoprtion/ionization, secretome, microfluidics, control theory**

I. Purpose

THE budding yeast *S. cerevisiae* serves as the model system as it is extensively well characterized and has an involved cellular regulatory system akin to many human signaling systems. We hope to demonstrate induced control of the biological system through simultaneous modifications to multiple system variables in order to gain a more advanced view of the nonlinearity and complexity present within cellular and molecular mechanisms. We focus on identifying yeast-specific gene transcriptional regulators that can be controlled via nano-bioreactor conditions and studied through a multiple-input/multiple-output (MIMO) feedback loop.

II. Experimental Design

For the majority of the experiments, we work with the standard wild-type *BY4741* yeast strain and make secretome measurements from intact yeast.

We begin with a large culture, stocked in frozen aliquots, assuring each generation of yeast and bud index is identical. Following agar plating and three days of growth at 30°C, we subculture a single colony in synthetic minimal media (SD), a defined cocktail with an addition of seven amino acids.

Parameters we look to adjust are glucose and galactose concentration to gain insight as to whether the *Gal4* transactivator can be regulated through conditioned media. We create subcultures at 0.1% glucose (*Gal4* activated), 2% glucose (repressed) and alternate to observe change and reversibility. Additionally, we introduce 2% galactose with 0.1% glucose to look at *Gal4* within the derepressed state.

Experimental set-up follows in a three-part sequence. MTNPs, containing approximately 9,000 cell traps 8-12 μM in length, are primed and loaded with the yeast cells through peek tubing into the device inlet [1]. They are perfused with desired media for four hours as time-lapsed images are captured every three minutes in order to measure cell proliferation. Peek tubing from the device outlet transfers the effluent containing secretome to a programmed Fab@Home x,y,z-robot that spots the excess liquid every five minutes on a matrix-assisted laser desoprtion/ionization (MALDI) sample plate. Analysis with mass spectrometry (MS) and MALDI MS Imaging Tool© allows us to examine temporal variation in peaks of interest identified by negating peaks that are present in the cellular media itself.

Future experiments will look at identifying further controllable transcriptional regulators controlled by sucrose, phosphate, sulfur and nitrogen levels; environmental stresses such as heat, oxidative stress, high salt concentration and pH; and the addition of a mating pheromone alpha factor.

III. Results

Preliminary results of yeast cell culture grown in SD and perfused with only water have shown three interesting peaks that discernibly increase in abundance over time, showing promise in our methods.

IV. Discussion

Typically, biological systems have been examined by changing a single parameter and holding all other conditions constant. The cellular regulatory system is an overly complex cycle [2] to be studied with simple yes-no experiments. We hope to use our research to demonstrate the feasibility of using a MIMO nonlinear system to model biological complexity and introduce control theory as an important aspect of future biological research.

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