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External Control of the \textbf{GAL Network} in \textit{S. cerevisiae}: A View from Control Theory

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Abstract

While there is a vast literature on the control systems that cells utilize to regulate their own state, there is little published work on the formal application of control theory to the external regulation of cellular functions. This paper chooses the GAL network in \textit{S. cerevisiae} as a well understood benchmark example to demonstrate how control theory can be employed to regulate intracellular mRNA levels via extracellular galactose. Based on a mathematical model reduced from the GAL network, we have demonstrated that a galactose dose necessary to drive and maintain the desired GAL genes’ mRNA levels can be calculated in an analytic form. And thus, a proportional feedback control can be designed to precisely regulate the level of mRNA. The benefits of the proposed feedback control are extensively investigated in terms of stability and parameter sensitivity. This paper demonstrates that feedback control can both significantly accelerate the process to precisely regulate mRNA levels and enhance the robustness of the overall cellular control system.

Introduction

The complexity of nested feedback control loops associated with complex biological systems creates many challenges to understanding biological systems. At the same time, the need for precise control of gene expression is increasing in fields such as genetics, cell and molecular engineering, and in disease treatment via gene expression control. A straightforward application for control of gene expression might be to generate a maximum output in bioreactor-based systems. The outputs from this type of application would typically by specific proteins of interest for biopharmaceuticals. The approach can be further applied to create a system in which the control of a gene network may be coupled with the expression of a recombinant protein.

On the other hand, systems biologists have built numerous mathematical models for gene and protein networks that cells utilize to control themselves [1]. The applications of such models have gone beyond simple qualitative understanding of network dynamics. Some models have been used in synthetic biology to affect metabolism and eventually control the biological processes toward desired outputs [2]. The full advantages of control theory, however, have not been realized, and there is little work on external control of cellular functions such as gene expression.

From a control theory perspective, control techniques used for complex engineering systems should in principle be applicable to the regulation of cellular systems. There are in general two potential challenges. First, it is not easy to find simple mathematical models for cellular system control. Most existing cellular mathematical models are not formulated in a standard control system framework. Typically, they lack global feedback loops from outputs to dynamically adjust the control dosage. Second, model parameters are usually very sensitive due to large uncertainties. The resulting control algorithm often becomes fragile, and the control system can remain stable only under small disturbances. Open-loop constant controls – for example, the gene knockdown or overexpression technique for yeast performed without adjusting dosages in real-time in response to the observed changes – are vulnerable to disturbances and individual heterogeneity.

It is desirable to have model-based, fine-tuned external control approaches to precisely regulate a reverse-engineered target. Unfortunately, achieving a reliable feedback control remains a challenge for cellular systems. Recently, Cantone \textit{et al.} developed the first reverse-engineering benchmark system [3], which is based on the GAL network using real-time image feedback. Control engineers have begun to apply closed-loop control ideas based on this benchmark [4,5].

In this paper we have employed model reduction techniques to simplify the complex GAL network, and further demonstrate that the simplified model is favorable for control system design. We also present the benefit of closed-loop system design. Our goal is to demonstrate the usefulness of control theory for precise external regulation of cellular systems and to open discussions in the field about possible future benefits of more advanced theoretical control.

The \textit{GAL} network in \textit{Saccharomyces cerevisiae}, is one of the most studied gene networks, and hence is well suited to serve as a demonstration, benchmark system. Galactose utilization in the
network can be broken up into four distinct stages based on proteins present in the cytoplasm. In the first stage $GAL2p$, the galactose transporter protein, allows galactose to enter into the cytoplasm [6]. Once internalized, $GAL3p$ binds to the cytoplasmic galactose and is activated. This causes the galactose to bind and inhibit the action of $GAL80p$ [7,8]. With $GAL80p$ bound, $GAL4p$ is able to activate the transcription of $GAL2$, $GAL3$, and $GAL80$ genes. In the presence of glucose, $GAL1p$ is also inhibited, which leads to inhibition of $GAL1$ expression [9]. Without accounting for the glucose network, the $GAL$ system is composed of two positive feedback loops, $GAL3p$ and $GAL2p$, and two negative feedback loops, $GAL80p$ and $GAL1p$. The glucose network proteins bind upstream $GAL1$, $GAL3$, and $GAL4$, and suppress transcription through the action of Mig1. The glucose network also prevents the binding of $GAL2p$ and external galactose and shuts down the galactose transportation. A mathematical model of the galactose network has previously been established by de Atauri et al. [10] and Ramsey et al. [11]. Bennett et al. combined a simplified glucose network with the galactose network model [12], and identified the model parameters using experimental data. A diagram of the simplified glucose and galactose networks used in this study is shown in Figure 1.

The key factor in designing a control system for a complex network that can be controlled externally is to design the system with measurable input(s) and output(s). For our study of the $GAL$ network, the measurable inputs into the system are the concentrations of galactose and glucose. The most common network, the measurable inputs into the system are the $GAL$ network and the glucose network, and reduces the sensitivity analysis successfully separates the galactose utilization network and the glucose network, and reduces the $GAL4p$ dimerization (the dotted lines) can be eliminated from the original model to create a reduced model. doi:10.1371/journal.pone.0019353.g001

![Figure 1. The gene regulatory network for galactose utilization (redrawn from Bennett et al. (2008) [12]). The extracellular inputs are $[gal]_e$ and $[glu]_e$. The natural output of the network is $a$ group of proteins. The $GAL4$ protein $g_4$ binds to upstream activation sites and activates the regulatory genes in the galactose network. The $GAL80$ gene inhibits the inducing effects of $GAL4$ and thereby provides negative feedback in the system. $GAL3$ enhances expression of $GAL4$ by binding with internal galactose (Gal), forming a $GAL3$-galactose complex, $g_3^*$, that inactivates $g_60$ by binding to it and resulting in a complex $g_{60}^-$. In addition, the transporter $GAL2$ increases the amount of internal galactose, which stimulates the galactose network. We use the $GAL1$ mRNA level, $m_1$, as the measureable output to control the system. According to sensitivity analysis, the interaction loops involving $GAL4p$ dimerization (the dotted lines) can be eliminated from the original model to create a reduced model.](image-url)
galactose is given, instead of manually tuning galactose by trial and error. Feedback control is introduced to improve the robustness of the controlled system and enhance the convergence rates. The simulation results show that both controls can achieve the control objective, i.e., maintaining GAL1 mRNA at a desired level. Similar analytic galactose dosages can be achieved for all the other measurable outputs, such as GAL2, GAL3, and GAL80. The difference between the open-loop control of the system with constant control input, and feedback control with time-dependent parameters, is that the feedback control significantly shortens the time required to achieve the steady state. The feedback loop indirectly increases the degradation rate of the internal galactose so that the system more rapidly reaches balance. The feedback control also significantly reduces parameter sensitivity. Feedback control takes advantage of information about the system state to regulate output, and in doing so significantly decreases the local sensitivity of measurements to the system parameters. Hence feedback control can resist much larger parameter perturbations/uncertainties as compared to the constant open-loop control; that is, an open-loop control with constant control input.

Methods

Mathematical Model for Yeast GAL Network

The GAL network is a good starting point to demonstrate feedback and feed forward external controls for cellular systems. Though simple, it is well understood and is easily manageable to interpret experimental results. On the other hand, it is complex enough to test sophisticated control algorithms. As illustrated in Figure 1, a mathematical model for the GAL network has been proposed based on the interactions between proteins and internalized galactose. The figure shows a gene regulatory network for galactose utilization (redrawn from Bennett et al. [2008] [12]). The extracellular inputs are [gal], and [glu]. The natural output of the network is a group of proteins. The GALA protein g1 binds to upstream activation sites and activates the regulatory genes in the galactose network. The GAL30 gene inhibits the inducing effects of GALA and thereby provides negative feedback in the system. GAL3 enhances expression of GAL4 by binding with internal galactose (Gal), forming a GAL3-galactose complex, g1*, that inactivates g80 by binding to it and resulting in a complex g80*. In addition, the transporter GAL2 increases the amount of internal galactose, which stimulates the galactose network. We use the GAL1 mRNA level, m1, as the measurable output to control the system. According to sensitivity analysis, the interaction loops involving GAL4p dimerization (the dotted lines) can be eliminated from the original model to create a reduced model. In the figure, g1 is the number of the galactose network protein monomers (i = 1, 2, 3, 4, 80), m1 is amount of mRNA (i = 1, 2, 3, 4, 80), g4d is the number of protein dimers (i = 4, 80), g4d is the number of GAL3p proteins bound to galactose, g4d is the number of GAL4p dimers bound to Gal80p dimers, and g80 is the number of GAL80p proteins bound to the Gal3p-galactose complex.

A complete mathematical model of the above gene network, which we term the Original model, can be described with 22 mathematical equations [12]. The first five represent the mass-action kinetics of galactose protein monomers, including dimerization, and the interaction with the internal galactose:

\[ \dot{g}_1 = \beta_1 m_1 - \delta_1 g_1 \]  
\[ \dot{g}_2 = \beta_2 m_2 - \delta_2 g_2 \]  
\[ \dot{g}_3 = \beta_3 m_3 - \delta_1 g_3 - k_3 x_{gal} g_3 + k_{-3} g_3 \]  
\[ \dot{g}_4 = \beta_4 R(x_{glu}) - \delta_1 g_4 - 2 k_{4d} g_4^c + 2 k_{-4d} g_{4d} \]  
\[ \dot{g}_{80} = \beta_{80} R(x_{glu}) - \delta_1 g_{80} - k_{80g1} g_{80}^c g_1 + k_{-80g1} g_{80}^c g_1 + 2 k_{-80g}_{80} - 2 k_{80} g_{80}^c g_{80} \]  

Equations (6)–(9) describe mRNA kinetics accounting for the transcription and translation of the galactose genes as well as the degradation of mRNA:

\[ \dot{m}_1 = x_1 R(x_{glu}) F(g_{4d}, g_{80}, A) - \gamma_1 m_1 - \frac{v_{ad} m_1 x_{glu}}{k_{ad} + m_1} \]  
\[ \dot{m}_2 = x_2 F(g_{4d}, g_{80}, 2) - \gamma_2 m_2 \]  
\[ \dot{m}_3 = x_3 R(x_{glu}) F(g_{4d}, g_{80}, 1) - \gamma_3 m_3 - \frac{v_{ad} m_3 x_{glu}}{k_{ad} + m_3} \]  
\[ \dot{m}_{80} = x_{80} F(g_{4d}, g_{80}, 1) - \gamma_{80} m_{80}. \]  

Equations (10)–(14) represent the kinetics of protein dimers and the associated complexes:

\[ \dot{g}_{4d} = k_{4d} g_4^c - k_{-4d} g_{4d} - k_{r1g80} g_{4d} + k_{-r1g80} - \delta_1 g_{4d} \]  
\[ \dot{g}_{80d} = k_{80g1} g_8^c g_1 - k_{80g1} g_{80d} - k_{-80g1} g_{80d} + k_{-80g1} - \delta_1 g_{80d} \]  
\[ \dot{g}_{4d}^c = k_{1g1} g_{13} + k_{-1g1} g_{13} + k_{80g1} g_{80}^c g_1 + k_{-80g1} g_{80}^c g_1 + \delta_1 g_{4d}^c \]  
\[ \dot{g}_{80} = k_{80g1} g_{80}^c - k_{-80g}_{80} - \delta_1 g_{80}^c. \]  

The metabolic reactions and transport of galactose are represented by a single equation:

\[ [\text{gal}] = R(x_{glu}) T_1 ([\text{gal}], [\text{gal}]) - \frac{k_{gal} [\text{gal}]}{k_{mpk} + [\text{gal}]} - k_{3g1} [\text{gal}] + k_{3g1} [\text{gal}] + k_{-3g1} - \delta_1 [\text{gal}]. \]  

Equations (16)–(18) describe a simplified glucose network, including the glucose-mediated enzymatic decay of GAL1 and GAL3 mRNA:
measured for intact cells, or by means of a microarray or other experimentally using a GFP conjugated to interest. The mRNA level of this target gene can be measured processes of the galactose network is represented as follows, respectively. They compose a simplified glucose network. The inhibitory function describing the number of upstream activation sites are the transport rates of external galactose and external glucose where $m_{\text{glu}}$, $x_{\text{glu}}$ and $[\text{glu}]$ are the amount of glucose network mRNA, associated protein, and cellular internal glucose, respectively. They compose a simplified glucose network. The inhibitory effect due to products of the glucose network that act on various processes of the galactose network is represented as follows,

$$R(x_{\text{glu}}) = \frac{\rho^g}{\rho^g + \gamma^g_{\text{glu}}}.$$  \hspace{1cm} (19)

where

$$T_1([\text{gal}]_e,[\text{gal}]_c) = \frac{[\text{gal}]_c - [\text{gal}]}{k_tr[gal] [\text{gal}]_c + [\text{gal}]_c + [\text{gal}]_e}$$ \hspace{1cm} (20)

and

$$T_2([\text{glu}]_e,[\text{glu}]_c) = \frac{[\text{glu}]_c - [\text{glu}]}{k_tr[glu] [\text{glu}]_c + [\text{glu}]_c + [\text{glu}]_e}$$ \hspace{1cm} (21)

are the transport rates of external galactose and external glucose into the cell, respectively. The cooperative fractional saturation function describing the number of upstream activation sites occupied on a promoter, assuming that $N$ sites exist [10], is given by

$$F([\text{gal}]_e,[\text{gal}]_c, N) = \frac{\sum_{i=0}^{N} \left( \begin{array}{c} N \\ i \end{array} \right) \left( K_{\text{gal}} [\text{gal}]_c \right)^i \left( K_{p1} [\text{gal}]_e \right)^{N-i} \left( K_{p2} [\text{gal}]_c \right)^h}{\sum_{i=0}^{N} \left( \begin{array}{c} N \\ i \end{array} \right) \left( K_{\text{gal}} [\text{gal}]_c \right)^i \left( K_{p1} [\text{gal}]_e \right)^{N-i} \left( K_{p2} [\text{gal}]_c \right)^h}.$$ \hspace{1cm} (22)

The above mathematical model is mainly based on the biochemical reactions occurring throughout the network. This approach of modeling the intermediates in a pathway has been widely used in both chemistry and biology. The variables and parameters used for the model are defined and summarized in Tables 1 and 2.

Our goal for galactose network control is to regulate the GAL family mRNA level by manipulating galactose and glucose concentrations. In this paper, we select GAL1 as the gene of interest. The mRNA level of this target gene can be measured experimentally using a GFP conjugated to Gal1, which can be measured for intact cells, or by means of a microarray or other assay that requires lysing a small fraction of the cells under observation. In practice, other reporter genes may be used to monitor Gal1 expression, for example, the red fluorescent protein from the gene dsRed [16]. In addition, it is notable that glucose suppresses all GAL genes [17]. External glucose must be kept at a very low level, i.e., $[\text{glu}]_e \approx 0$, or the Gal network will be inhibited. Once no external glucose is supplied, the galactose network will soon reach its steady state, which means one can focus on the galactose network.

From a control engineering perspective, one key step is to identify inputs (control) and outputs (preferably measurable), so that the system can be put into a control framework for discussion. $[\text{gal}]_e$ and $[\text{glu}]_e$ external galactose and glucose levels, are the control variables in this study, while the mRNA levels of GAL1, GAL2, GAL3, and GAL80, $m_1$, $m_2$, $m_3$, $m_9$, are measurable variables. The goal of this research was to design a time-course of $[\text{gal}]_e$ such that one of the mRNAs $m_1$, $m_2$, $m_3$, $m_9$ is maintained at a desired level.

While there are several methods that might be suitable for culturing yeast under external control, microfluidic devices would be ideal for experimental implementation of the cellular control system [18]. Fluorescence microscopy could be used to quantify, in real-time, the levels of GFP-labeled Gal1p from a small, synchronized population of S. cerevisiae, with the output of a camera or photomultiplier tube serving as the sensor measurement to the control system. Ion mobility-mass spectrometry could also be used to monitor the secreted metabolites and signaling molecules in real-time and intracellular species after cell lysis [19]. Microfluidic valves could control the concentrations of various chemicals and serve as the control system outputs.

In the next section, we will conduct a sensitivity analysis to simplify this subsystem. As shown in Figure 1, the interaction loops involving Gal1p dimerization can be removed. The reduced

### Table 1. Model Variables.

| Variable | Description | Initial value (molec.) [11] |
|----------|-------------|-----------------------------|
| $g_1$ (molec./cell) | Gal1p | 132.3 |
| $g_2$ (molec./cell) | Gal2p | 1156.7 |
| $g_3$ (molec./cell) | Gal3p | 4341.2 |
| $g_4$ (molec./cell) | Gal4p | 0.1563 |
| $g_{80}$ (molec./cell) | Gal80p | 0.1138 |
| $m_1$ (molec./cell) | GAL1 mRNA | 0.2647 |
| $m_2$ (molec./cell) | GAL2 mRNA | 0.3305 |
| $m_3$ (molec./cell) | GAL3 mRNA | 0.9044 |
| $m_9$ (molec./cell) | GAL80 mRNA | 1.1871 |
| $u_1$ (molec./cell) | Gal4p dimer | 308.92 |
| $u_{80}$ (molec./cell) | Gal80p dimer | 157.229 |
| $e_1$ (molec./cell) | Gal1p dimer - Gal80p dimer complex | 0 |
| $e_2$ (molec./cell) | Gal3p – gal. complex | 0 |
| $e_3$ (molec./cell) | Gal80p – Gal3p – Gal. complex | 0 |
| $[\text{gal}]_e$ (molec./cell) | Internal galactose | 0 |
| $m_{80}$ (molec./cell) | Glucose network mRNA | 4000 |
| $x_{80}$ (molec./cell) | Glucose network proteins | 15000 |
| $[\text{glu}]_e$ (molec./cell) | Internal glucose | 150000 |
| $m_{9}$ (molec./cell) | External galactose outside the cell | $0 \sim 2.366 \times 10^8$ |
| $m_{9}$ (molec./cell) | External glucose outside the cell | $0 \sim 2.957 \times 10^8$ |

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Table 2. Model Parameters.

| Parameter | Description | Value [12] |
|-----------|-------------|------------|
| $f_1$ (min$^{-1}$) | Translation rate of Gal1p $g_1$ | 9.92 |
| $f_2$ (min$^{-1}$) | Translation rate of Gal2p $g_2$ | 6.94 |
| $f_3$ (min$^{-1}$) | Translation rate of Gal3p $g_3$ | 18.0 |
| $f_4$ (molec./cell)/min | Max translation rate of Gal4p $g_4$ | 0.86 |
| $f_{80}$ (min$^{-1}$) | Translation rate of Gal80p $g_{80}$ | 4.00 |
| $s_1$ (molec./cell)/min | Max translation rate of GAL1 | 1.09 |
| $s_2$ (molec./cell)/min | Max translation rate of GAL2 | 1.20 |
| $s_3$ (molec./cell)/min | Max translation rate of GAL3 | 36.0 |
| $s_{80}$ (molec./cell)/min | Max translation rate of GAL80 | 3.00 |
| $\delta_1$ (min$^{-1}$) | Deg. rate of gal. proteins and complexes | 0.0033 |
| $\gamma_1$ (min$^{-1}$) | Deg. rate of GAL1 mRNA | 0.036 |
| $\gamma_2$ (min$^{-1}$) | Deg. rate of GAL2 mRNA | 0.026 |
| $\gamma_3$ (min$^{-1}$) | Deg. rate of GAL3 mRNA | 0.036 |
| $\gamma_{80}$ (min$^{-1}$) | Deg. rate of GAL80 mRNA | 0.036 |
| $k_3$ (molec./cell)/min | Binding rate of $g_3$ to $x_{gal}$ | $5.0 \times 10^{-8}$ |
| $k_{-3}$ (min$^{-1}$) | Dissociation rate of $g_3$ | 890 |
| $k_{4d}$ (molec./cell)/min | Binding rate of $g_4$ to $g_4$ | 0.1 |
| $k_{-4d}$ (min$^{-1}$) | Dissociation rate of $g_{4d}$ | 1.0 |
| $k_{80d}$ (molec./cell)/min | Binding rate of $g_{80}$ to $g_{4d}$ | 0.10 |
| $k_{-80d}$ (min$^{-1}$) | Dissociation rate of $g_{80d}$ | 170 |
| $k_{800d}$ (molec./cell)/min | Binding rate of $g_{80}$ to $g_{800d}$ | 0.10 |
| $k_{-800d}$ (min$^{-1}$) | Dissociation rate of $g_{800d}$ | 0.03 |
| $k_{3}$ (molec./cell)/min | Binding rate of $g_3$ to $g_{80}$ | 0.10 |
| $k_{-3}$ (min$^{-1}$) | Dissociation rate of $g_{3}$ | 1.80 |
| $k_{gal}$ (molec./cell) | Galactose metabolism rate | 3350 |
| $k_{mgk}$ (molec./cell) | Galactose metabolism constant | $1.29 \times 10^7$ |
| $k_{tr}$ (min$^{-1}$) | Galactose transport rate | 4350 |
| $k_{mtr}$ (molec./cell) | Galactose transport constant | $2.15 \times 10^8$ |
| $\alpha_g$ (unitless) | Galactose interactive constant | 10.0 |
| $K_p$ (cell/molec.) | Equilibrium constant of $g_4$ binding to UAS | 0.091 |
| $K_q$ (cell/molec.) | Equilibrium constant of $g_{80}$ binding to $g_{4d}$ | 0.0556 |
| $C_T$ (unitless) | Cooperative binding constant of $g_{4d}$ to UAS | 1 |
| $C_{U}$ (unitless) | Cooperative binding constant of $g_{80}$ to $g_{4d}$ -UAS complexes | 30 |
| $\sigma_{glu}$ (molec./cell)/min | Basal transcription rate of glucose DNA | 215 |
| $\sigma_{glu}$ (molec./cell)/min | Activated transcription rate of glucose DNA | $6.452 \times 10^3$ |
| $\sigma_{glu}$ (min$^{-1}$) | Translation rate of glucose proteins | 0.4 |
| $\delta_{glu}$ (min$^{-1}$) | Degradation rate of glucose proteins | 0.1 |
| $\gamma_{glu}$ (min$^{-1}$) | Degradation rate of glucose mRNA | 0.0633 |
| $\delta_{glu}$ (min$^{-1}$) | Dilution rate of glucose | 0.0033 |
| $\kappa_{glu}$ (molec./cell) | Hill constant for glucose induction | $1.075 \times 10^3$ |
| $b$ (unitless) | Hill coefficient for glucose induction | 1.8 |
| $k_{ir2}$ (min$^{-1}$) | Glucose transport rate | 4350 |
| $k_{mtr2}$ (molec./cell) | Glucose transport constant | $6.022 \times 10^8$ |
| $\alpha_{glu}$ (unitless) | Glucose interactive constant | 1.0 |
| $\beta_{glu}$ (min$^{-1}$) | Glucose transport rate | 5350 |
| $K_{glu}$ (molec./cell) | Glucose transport constant | $1.29 \times 10^7$ |
| $p$ (molec./cell) | Hill constant for gal. repression | $1.29 \times 10^7$ |
| $q$ (unitless) | Hill coefficient for gal. repression | 0.8 |
model lowers the complexity to design the associated controller, allowing analytical implementation of a feasible design. We will show later that the reduced model may cause bounded deviation from the original model in transient response; however, it results in insignificant deviation from the steady states.

Model Reduction

Given the steady states of the glucose utilization network, the original model still has 35 parameters. The large number of parameters and significant nonlinearities make it difficult to design an effective controller. As with any biological system, the number of parameters involved in the induction of a specific pathway could be large, with physical, chemical, and biological parameters all affecting the system. In terms of a modeling-based representation of biological systems, the goal of our effort is to determine the parameters that most affect the induction of the system.

A simple example of this concept can be observed in glycolysis. Through the complex intermediates and interactions within this pathway, three regulatory steps are often considered, biologically, the most important rate-limiting steps in the pathway. These irreversible steps, phosphorylation of glucose, phosphorylation of fructose-6-phosphate, and transfer of phosphate to phosphoenolpyruvate and then to ADP, are the most important control points. The proteins involved in these steps, as well as the mRNA that generates these proteins, are crucial to the overall process. Investigating the influence of the parameters on the control target is an effective tool to reduce the model complexity and assist control system design. In order to reduce the model, we should know whether the output significantly changes under a small change of the parameters, as some parameters may not be as effective as others. By understanding how small changes in parameters affect the overall system, we can determine the most important parameters involved in generating the desired output.

In the case of GAL1 mRNA, a small change in the value of the GAL4p parameter will have a large effect on GAL1 mRNA expression, indicating that this parameter significantly influences the uncertainties of GAL1 mRNA expression. Thus, this parameter is crucial to control the system. Small changes in a parameter that was not as closely linked to GAL1 mRNA expression specifically may not have a large effect on the expression. In fact, most biological systems may be sensitive to certain parameters over a large range. If the sensitivity of a parameter is small compared to other parameters, the parameter could be arbitrarily chosen from a wide range of values without altering the dynamic performance of the system. This practice has been used successfully in control systems engineering, including chemical process control, robotics control, aerospace control, etc.

For the above model, we will first conduct local sensitivity analysis that will quantify the change of the system states due to constant perturbation of a parameter at time zero. Consider a general form of nonlinear ordinary differential equations:

$$\frac{dX}{dt} = f(X, \phi, t), \quad X(t_0) = X_0,$$

where $X$ is the state vector with $n$ components, and $\phi$ is the parameter vector with $m$ components. The initial condition of the above equations is set to $x_0$. Define the sensitivity coefficients of the state $x_i$ with respect to parameter $\phi_j$, $j=1, \ldots, m$, by

$$s(x_i; \phi_j, t) = \frac{\partial x_i}{\partial \phi_j},$$

and then the state’s sensitivity trajectory can be described by the following:

$$\frac{ds(X; \phi, t)}{dt} = \frac{d(\partial X/\partial \phi_j)}{dt} = \frac{\partial f}{\partial x} \frac{\partial X}{\partial \phi_j} + \frac{\partial f}{\partial \phi_j}$$

where the state vector is denoted by $X = [x_1 \ldots x_n]^T$. The initial values of the sensitivity equations can be obtained by

$$s(x_i; \phi_j, t_0) = \frac{\partial x_i}{\partial \phi_j} = \begin{cases} 0, & x_i \neq \phi_j \\ 1, & x_i = \phi_j \end{cases}.$$

In order to avoid scale differences in the parameters, the sensitivity coefficients are normalized as follows,

$$ns(x_i; \phi_j, t) = \frac{\partial x_i}{\partial \phi_j}.\frac{x_i}{\phi_j}.$$

The normalized sensitivity coefficient $ns(x_i; \phi_j, t)$ means that a 1 percent change of state $x_i$ results in a 1 percent change of the parameter $\phi_j$ over the time course $t$. The sensitivity trajectory $s(x; \phi_j, t)$ can be calculated numerically by equations (25–26). The importance of the parameters can thus be distinguished by the magnitude of the normalized sensitivity coefficients in the time-course of the system following the constant perturbation at time zero.

The normalized sensitivity coefficients for the original model are shown in Figure 2, where each small block describes a normalized sensitivity for a certain parameter at a certain time. In Figure 2A, the galactose input is kept at a constant,
[gal]_e = 4 \times 10^7 \text{ (molec.)}

with the measured system output represented by m_1. It is easy to observe that the external galactose input is positively correlated to the output of m_1. The glucose network remains in the steady state when the internal glucose is emptied and there is no external glucose supply. The steady state of [glu] = 13586 (molec./cell) and m_phi = 3397 (molec./cell) can be determined by solving m_phi = g_phi = [glu] = 0. The function R([glu]) in equation (19) was found to equal 0.9959. Thus, the glucose network can be separated from the galactose network. This is in agreement with experimental data, where a lack of glucose as a carbon source and the presence of galactose will induce the GAL network. Based on the sensitivity analysis of m_1 with respect to all the parameters (Table 2), it becomes clear which parameters can be omitted from the system. The heat map in Figure 2 shows the sensitivities of the parameters of the parameters gal

GAL normalized sensitivity of m_4

determined by solving m_phi = g_phi = [glu] = 0. The function R([glu]) in equation (19) was found to equal 0.9959. Thus, the glucose network can be separated from the galactose network. This is in agreement with experimental data, where a lack of glucose as a carbon source and the presence of galactose will induce the GAL network. Based on the sensitivity analysis of m_1 with respect to all the parameters (Table 2), it becomes clear which parameters can be omitted from the system. The heat map in Figure 2 shows the sensitivities when [gal]_e = 4 \times 10^7 \text{ molec.}

The sensitivities of the parameters δ_1, α_1, γ_1, β_1, k_gal, k_glu, k_b, k_r, K_p, C_p and K_gal are much smaller than those of the other parameters (near zero, green color). In general, when the external galactose [gal]_e is higher, the parameters are more sensitive, with the exception of δ_1 and γ_1.

However, it would be careless to omit these parameters without further investigation. For example, the degradation rate δ_1 is important for achieving the steady states of the original model, so it must be retained. K_p and C_p come from the cooperative fractional saturation function F(g_a,t,s_gal,N). Both g_a,t and g_b,t in the function contribute to the activation of mRNAs m_1, m_2, m_3 and m_4. However, the contributions may not be equal. If we define P = C_pK_gal; Q = C_bK_gal, then we have

F(g_a,t,s_gal,1) = \frac{P}{1 + P + PQ} = \frac{1}{1 + 1 + Q} = 1 - \frac{Q}{1 + Q} (28)

From (29), we observed that the output m_1 is positively correlated to the external galactose input. The sensitivity analysis has told us that K_p and C_p cannot be set to be large enough to satisfy 1 + Q >> 1/P. Thus, only g_b,t contributes to the cooperative

Figure 2. Normalized parameter sensitivity trajectory with respect to time. The heat map shows the normalized sensitivity of GAL1 mRNA concentration (m_1) towards change in all 35 parameters. Panel (A): Sensitivities when [gal]_e = 4 \times 10^7 \text{ molec.} Panel (B): Sensitivities when [gal]_e = 4 \times 10^7 \text{ molec.} The sensitivities of the parameters δ_1, α_1, γ_1, β_1, k_gal, k_glu, k_b, k_r, K_p, C_p and K_gal are much smaller than those of the other parameters (near zero, green color). In general, when the external galactose [gal]_e is higher, the parameters are more sensitive, with the exception of δ_1 and γ_1.

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fractional saturation function. Similarly, the other two cooperative functions can be simplified as follows,

\[
F(g_{4d}, g_{80d}, 2) = \frac{2P + P^2 + 2PQ}{1 + 2P + P^2 + 2PQ + 2PQ + P^2Q^2} \\
\approx 1 - \left( \frac{Q}{1+Q} \right)^2, \text{ if } 1 + Q > \frac{1}{P},
\]

and

\[
F(g_{4d}, g_{80d}, 4) \approx 1 - \left( \frac{Q}{1+Q} \right)^4, \text{ if } 1 + Q > \frac{1}{P}.
\]

Therefore, the cooperative fractional saturation effect of \(g_{4d}\) and \(g_{80d}\) can be approximated by the contribution from \(g_{80d}\) alone. This can also be explained by the sensitivity analysis in Figures 1, 3, and 4, which shows that \(k_{4d}, k_{-4d}, k_r\), as well as \(k_r\), are less “essential” to the system output. Thus, we can set \(k_{4d}, k_{-4d}, k_r,\) \(k_{-r}\), \(k_{-r}\), \(k_{-r}\), \(k_{-r}\), \(k_{-r}\), \(k_{-r}\), and \(C_p\) are smaller than 0.25, while the others are larger than 1. This implies the reaction terms associated with insensitive parameters can be omitted no matter which mRNA is chosen as the control target. One can conclude not only that the control model targeting GAL1 mRNA need not include the terms associated with the above parameters, but also that control strategies targeting all four GAL mRNAs can suppress the same terms. The reduced model can thus be used for the control design targeting any of the four GAL mRNAs.

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Thus, a Reduced Order Model can be achieved based on the analysis above. The Reduced Order Model in a state space form can thus be described as follows:

\[ \dot{g}_1 = \beta_1 m_1 - \delta_1 g_1 \]  
\[ \dot{g}_2 = \beta_2 m_2 - \delta_1 g_2 \]  
\[ \dot{g}_3 = -\delta_1 g_3 - k_3 x_{gal} g_3 + k_{-3} g_3^2 + \beta_3 m_3 \]  
\[ \dot{g}_{80} = \beta_{80} m_{80} - \delta_1 g_{80} - k_{80} g_{80}^2 + k_{-80} g_{80} e_{gal} + k_{-80} g_{80}^2 g_{80} + k_{-80} g_{80} e_{gal}^2 - \delta_1 g_{80}^2 \]  
\[ m_1 = x_1 F(g_{80}, 4) - \gamma_1 m_1 \]  
\[ m_2 = x_2 F(g_{80}, 2) - \gamma_2 m_2 \]  
\[ m_3 = x_3 F(g_{80}, 1) - \gamma_3 m_3 \]  
\[ m_{80} = x_{80} F(g_{80}, 1) - \gamma_{80} m_{80} \]  
\[ \dot{g}_{80d} = k_{80d} g_{80}^2 - k_{-80d} g_{80d} - \delta_1 g_{80d} \]

where

\[ F(g_{80d}, N) = 1 - \left( \frac{C_p K_d g_{80d}}{1 + C_d K_d g_{80d}} \right)^N. \]

All parameters are summarized in Table 2.

The comparison of the outputs of the Original Model and the Reduced Model is shown in Figure 5. The differences between the two models occur in the transition process before the first 200 min, while the difference decreases significantly in the steady state. According to the experimental results from [11,12], the galactose network takes 4–7 hours [240–420 minutes] to reach a steady state. Our simulation results in Figure 3 are consistent with the experimental data. When the external galactose level is set at \([g_{gal}] = 4 \times 10^7\) molec. or \([g_{gal}] = 4 \times 10^5\) molec., the steady-state deviation of GAL1 mRNA, \(m_1\) (molec./cell) is similar between the full and reduced models. However, the deviation of the transition process, the time before achieving the steady state, between the two models with the smaller external galactose level is significant. This result is consistent with the sensitivity measure in Figure 3.
Biologically, the reduced model varies from the complex model only within the first 200 minutes of expression for relatively low galactose levels, 400 molecules. The steady-state levels of GAL1 mRNA expression after the first 200 min for both galactose levels tested were the same in both models (Figure 5). In this way, the reduced model can be used to explain the expression of GAL1 mRNA, once the expression level has reached a steady state. Before the steady state is reached, only a complex model can completely model the effects of GAL1 mRNA expression from the introduction of low levels of galactose. A similar technique can be applied if the control target changes to any of the other mRNAs or proteins. This paper exemplifies GAL1 mRNA as a primary control target, but the applications of the method are not limited to a certain mRNA. Choosing GAL2 or GAL3, as well as GAL80, will produce very similar reduced models; however, using GAL4 as a control target will obviously yield a different reduced model because of the simplification in the GAL1 output system obtained by the removal of the GAL4p subsystem. A reduced model for GAL4 would have to include the GAL4p subsystem, and thus a much different reduced model would be generated.

Figure 3 describes the local sensitivity analysis at the steady states of four GAL mRNAs with respect to all model parameters. The bars denote the range of the sensitivity coefficients of four GAL mRNAs with respect to each parameter. The parameters \( \delta_1, \alpha_{\text{act}}, \nu_{\text{act}}, k_{\text{act}}, \beta_4, k_{4a}, k_{-a}, k_4, k_p, \) and \( C_p \) are smaller than 0.25, while the others are greater than one. This implies that the reaction terms associated with the insensitive parameters can be omitted no matter which mRNA is chosen as the control target. The design of a control model to control steady-state expression of GAL1, GAL2, GAL3, and GAL80 mRNA (\( m_1, m_2, m_3, \) and \( m_8 \)) is described in the following section. The local sensitivity analysis describes the change of system dynamics at time \( t \) after a step-wise perturbation to a parameter occurs at time zero. One might wonder what the effect would be when all parameters are perturbed simultaneously. Global sensitivity analysis investigates the effect of simultaneous large variations of all parameters on the states. The common methods to test global sensitivity include the Fourier amplitude sensitivity test (FAST) [20], extended FAST [21], Sobol’s method [22], the partial rank correlation coefficient (PRCC) [23], as well as the weighted average local sensitivity method [24].

FAST is one of the classical methods to determine global sensitivity. Assuming that the time dependence of each uncertain parameter \( \phi_i \) is associated with a frequency \( \omega_j \), we can describe that parameter by a transformation function of a sinusoid, \( i.e., \)

\[
\phi_j(s) = G_j[\sin(\omega_j s)], \quad j = 1, 2, \ldots, m
\]

for all \( m \) parameters, where \( s \) is the time. The first order global sensitivity of \( x_i \) with respect to the variation of the parameter \( \phi_i \) can be defined as the ratio of the variance of \( \phi_i \) to the total variance

\[
GS_i(\phi_j) = \sigma_{\text{global}}^2 / \sigma_i^2
\]

where the total variance is

\[
\sigma_i^2 = 2 \sum_{k=1}^{\infty} [A_i^2(k) + B_i^2(k)]
\]
and the variance of $\phi_j$ becomes

$$\sigma^2 \phi_{ij} = \frac{1}{2\pi} \int_0^{2\pi} x_i(\phi,s) \cos(\phi) ds$$

(49)

$$B_i(\phi) = \frac{1}{2\pi} \int_{-\pi}^{\pi} x_i(\phi,s) \sin(\phi) ds.$$  

(50)

Although the above local sensitivity analysis pointed out some candidate parameters and their associated terms that may be removed, it only guarantees the scenario when the parameters are tuned one by one. When the parameters are simultaneously adjusted, some may become sensitive. Thus, we must also apply global sensitivity analysis to rule out corresponding parameters.

As shown in Figure 4, the bars denote the range of the sensitivity coefficients of four $GAL$ mRNAs with respect to each parameter under constant control $[g_{1\text{d}}]=4 \times 10^7$ mole. The blue solid line stands for the global sensitivity coefficients for $GAL1$ mRNA. In particular, $a_{1r}$, $k_{1d}$, $B_1$, $k_{1d}$, $k_{1q}$, $k_{r}$, $K_{p}$, and $C_p$ are nearly zero. The maximum of the coefficients $\delta_{1}, \nu_{1d}, k_d$ and $k_{80d}$ is smaller than 0.2, while that of the others is larger than 0.2, consistent with the local sensitivity coefficients in Figure 3. This implies that the reaction terms associated with insensitive parameters can be omitted without matter which mRNA is chosen as the control target. Thus, both local and global sensitivity analyses indicate that the reduced model, equations (32–43), can be used for regulating all four $GAL$ mRNAs.

**Control Design**

As discussed earlier, any of the four $GAL$ mRNAs can be chosen as the control target. For demonstration purposes, we have chosen to control $GAL1$ mRNA ($m_1$), i.e., essentially maintain the $GAL1$ mRNA at a desired level $m_1^*$, i.e.,

$$m_1 = m_1^* \text{ and } \dot{m}_1 = \dot{m}_1^* = 0.$$  

(51)

In order to keep equation (51) true, the steady state of the system becomes

$$g_{80d}^* = \frac{1}{C_p K_q} \left(1 - \frac{1}{C_p K_q} \right) \frac{1}{[1 - \gamma_1 m_1^*/x_1]^{3/4}}.$$  

(52)

As a test case for this model, we will demonstrate the control of $GAL1$ mRNA expression by controlling the external level of galactose. It is important to note that any gene or protein represented in the reduced model can be controlled in a similar manner. Following this same pattern, recombinant genes regulated by $GAL1$ could be controlled in the same manner.

Similarly, we can find the steady states for $m_2, m_3, m_{80}$, and $g_1$,

$$m_2^* = \frac{\gamma_2}{\gamma_3} g_{80d}^*$$  

(53)

$$m_3^* = \frac{\gamma_3}{\gamma_3} g_{80d}^*$$  

(54)

$$m_{80}^* = \frac{\gamma_{80d}}{\gamma_{80d}} g_{80d}^*$$  

(55)

$$g_1^* = \beta m_1^*/\delta_1$$  

(56)

$$g_2^* = \beta m_2^*/\delta_1$$  

(57)

$$g_{80}^* = \frac{\sqrt{g_{80d}}}{\sqrt{m_{80d}}/k_{80d}}$$  

(58)

$$g_{80}^* = \frac{(g_{80})^*}{(g_{80})^*}$$  

(59)

Observing the galactose network, we can obtain two auxiliary equations:

$$\frac{d(g_3 + (g_3)^*)}{dt} = \beta g_3 m_3 - \delta_1 (g_3 + (g_3)^*)$$  

(60)

$$\frac{d(g_{80} + 2g_{80d} + (g_{80})^*)}{dt} = \beta_{80} m_{80} - \delta_1 (g_{80} + 2g_{80d} + (g_{80})^*)$$  

(61)

Equation (60) indicates that the total mass of $(g_3, (g_3)^*, (g_80)^*)$, is up-regulated by $m_3$. Biologically, this is a simple concept to understand, as expression of $GAL3$ mRNA, $m_3$, is necessary for the generation of $GAL3$ p. $g_3$, and the Gal3p dimer, $(g_3)^*$, in a dose-dependent manner. Similarly, the $GAL30$ p/GAL3p dimer complex is dependent on the expression of $GAL3p$. Equation (61) indicates...
that the total mass of \((g_{80}, g_{80d}, g_{80a}^*)\), is up-regulated by \(m_{80}\), in a manner similar to that described above. Both of these equations show the interplay between the biological mechanisms responsible for their creation.

Through equations (60) and (61) we can determine the remaining steady states \(g_1^*, (g_{80}^*)^*\) and \([gal]^*\) using the auxiliary equations

\[
g_1^* + (g_1^*)^* + (g_{80}^*)^* = \beta_1 m_1^*/\delta_1
\]  
\[
g_80^* + 2g_{80d}^* + (g_{80}^*)^* = \beta_80m_{80}^*/\delta_1.
\]

Thus,

\[
(g_{80}^*)^* = \beta_80m_{80}^*/\delta_1 - m_{80}^* - 2g_{80d}^*
\]

\[
g_3^* = \beta_3 m_3^*/\delta_1 - (g_3^*)^* - (g_{80}^*)^*
\]

\[
[gal]^* = \frac{(k - \delta_1)(g_1^*)^* + \delta_1 (g_{80}^*)^*}{(k_3 g_3^*)}.
\]

Given our desire to enforce the control condition of \(m_1 = m_1^*\), we need to control the external galactose, \([gal]^*\), which at steady state can be calculated as follows,

\[
[gal]^* = \frac{k_{gal} \Omega + [gal]^* (1 + \Omega)}{1 - \Omega},
\]

where \(\Omega = \left(\frac{k_{gal} g_1^* [gal]^*}{k_{gag1} + [gal]^*} + k_3 g_3^* [gal]^* - k_{-3} (g_3^*)^* + \delta_1 [gal]^*\right)/ (k_{gag2} g_2^*)\).

Similarly, if the control objective is to regulate \(GAL2\) mRNA, \(m_2 = m_2^*\), then

\[
g_{80a}^* = \frac{1}{C_q K_q} \left[ \frac{1}{1 - \sqrt{1 - \gamma_2 / x_2 m_2^*}} - \frac{1}{C_q K_q} \right]
\]

\[
m_1^* = x_3 F(g_{80a}^*, 4) / \gamma_1.
\]

The rest of the terms can be achieved using the same equations (54)–(67). Because this system has only a single steady state, one can always find a nominal control value for external galactose \([gal]^*_c\) to maintain any control objective at a desired level. In other words, we can always compute the necessary amount of galactose offline in order to maintain \(GAL1\) mRNA or any other factor, such as \(GAL2\) mRNA or \(GAL3p\), at a level of interest.

Thus, offline, we can calculate an open-loop constant control, which is the simplest type of controller that does not take into consideration state information that is fed back into the system for control purposes.

\[
[gal]^*_c = [gal]^*_e
\]

for any of the targeting mRNA, \(m_1, m_2, m_3\), and \(m_{80}\) if these mRNAs are not measurable in real-time. However, if they are measurable, one can improve the control algorithm by introducing a closed-loop feedback control. A simple proportional output feedback control for \(m_1\) can be described as

\[
Closed-loop Feedback Control: [gal]^*_c = [gal]^*_e + k(m_1^* - m_1)
\]

where the feedback gain \(k = 10^5\). The gain \(k\) directly affects the convergence rate of the controlled GAL network. However, the external galactose amount should not exceed the maximum concentration of galactose. Thus, the control dosage should be bounded by

\[
[gal]^*_c = \min \left( [gal]^*_c, [gal]^*_c \right)
\]

Of course, one can use a proportional-integral-derivative (PID) control in practice. Integral control can adjust the static error, but too much integral gain can reduce the stabilization of the system. Derivative control can adjust the convergence rates, however, it is too sensitive to high frequency noise. For pursuing different requirements, many more complicated control methods can be applied, such as optimal control for minimizing the galactose dosage and robust control for reducing the uncertainty, among others. Because the control objective of regulating the mRNA concentration to a desired value, is a relatively simple task, without strict requirements for reaction time and galactose dosage, a simple proportional control is adequate to accomplish the control goal.

Results

Control Stability Analysis

Next, we will prove the stability of this equilibrium because the equilibrium is a unique steady state. If it is stable, then the state will tend to this steady state given the nominal control. The control objective \(m_1\) will be maintained at the desired level \(m_1^*\). Biologically, this system is stable based on experimental results [12]. The following is a theoretical proof based on the mathematical model.

The Jacobian matrix at this equilibrium is
systems finds that the equilibrium is asymptotically stable. Thus it implies that the convergence of this controlled system will take a long time. Biologically, after we set the external galactose level to a constant value, the yeast galactose utilization network will slowly achieve balance (a steady state) and the GAL1 mRNA level will gradually reach and maintain the desired level. At this steady-state level, there will not be oscillations in the mRNA or protein levels associated with this system.

In order to change the eigenvalues to negative, we follow a common practice in control systems engineering and introduce a linear output feedback control

\[
[j \text{al}]_r = [j \text{al}]^* + k(m^*_1 - m_1),
\]

where

\[ F_1 = -4\gamma_1(K_q C_q)^2(\frac{g^{\text{gal}}_{80d}}{(1 + K_q C_q g^{\text{gal}}_{80d})^2}), \]

\[ F_2 = -2\gamma_2(K_q C_q)^2 g^{\text{gal}}_{80d}/(1 + K_q C_q g^{\text{gal}}_{80d})^2, \]

\[ F_3 = -3K_q C_q/(1 + K_q C_q g^{\text{gal}}_{80d}), \]

\[ F_{80} = -80K_q C_q/(1 + K_q C_q g^{\text{gal}}_{80d}), \]

\[ G_1 = \frac{k_{\text{gal}}[j \text{al}]^*}{k_{\text{mgk}} + [j \text{al}]^*}, \]

\[ G_2 = k_{\text{gal}}^* - [j \text{al}]^*/k_{\text{mgk}} + [j \text{al}]^* + [j \text{al}]^*, \]

\[ G_3 = k_{3}g^{\text{gal}}_{80d} + \frac{k_{\text{gal}}g^{\text{gal}}_{80d}}{(k_{\text{mgk}} + [j \text{al}]^* + [j \text{al}]^*)} + \frac{(2[j \text{al}]^* + k_{\text{mgk}}}{{k_{\text{mgk}} + [j \text{al}]^*} + [j \text{al}]^*)^2. \]

If \( m^*_1 = 20 \), one finds the eigenvalues of the Jacobian matrix are \([-1895.99, -194.67, -12.54, -12.04, -0.0295, -0.0387+0.0024i, -0.0387-0.0024i, -0.0360, -0.0015, -0.0033, -0.0033, -0.0033] \). A standard stability analysis of the dynamic systems finds that the equilibrium is asymptotically stable. Thus the system will tend to the steady state asymptotically.

Because most of the eigenvalues are small in magnitude, it implies that the convergence of this controlled system will take a
The effects of protein concentration profiles by feedback control on GAL1 mRNA expression on the GAL network are illustrated in Figure 7. All states (unit: molec./cell) converge to a stable steady state, while \( g_2, g_3 \) and \( g_{\text{so}} \) have slower convergence rates than the other states. It is not surprising to see that all concentrations tend to a steady state, since the system has a unique stable steady state. But the transient response of the galactose system is equally important, because a large fluctuation is not desired in a reliable bioreactor. The figure shows that the system does not have large overshoots and is thus safe for implementation in practice.

Figure 8 compares the set point regulation performance of the feedback control between the original model and the reduced model using feedback control. Setting three desired levels of \( m_1 \) as 10, 15, and 20, feedback control based on both the original model (solid) and the reduced model (open) can be maintained at the desired levels within a similar time frame. The reduced model-based feedback control, however, leads to slightly larger magnitudes of oscillations at the transient response. The difference is small enough not to result in any severe fluctuation in the biological system. Thus, the control design based on the reduced model can be applied to the original model directly.

Sensitivity Analysis

The steady-state sensitivity describes the change of the steady state given a small perturbation of a certain parameter [25]. It can be given by the first-order partial derivative of a state with respect to the parameter. Using the finite difference method, the normalized steady-state sensitivity (NSSS) coefficient for the steady state of state \( x_i \) with respect to the parameter \( \phi_j \) can be approximated by

\[
SS_{ij} = \frac{\delta Z_{SS}(\phi_j) / Z_{SS}(\phi_j)}{\delta \phi_j / \phi_j} \approx \frac{Z_{SS}(\phi_j + \Delta \phi_j) - Z_{SS}(\phi_j)}{\Delta \phi_j},
\]

where \( Z_{SS} \) is the steady state of the state \( x_i \).

A small magnitude of NSSS of state \( x_i \) with respect to the parameter \( \phi_j \) implies that the parameter uncertainty of \( \phi_j \) has no significant influence on the state \( x_i \) locally. In contrast, a large NSSS implies serious influence by the parameter uncertainty. It is necessary to emphasize “locally” because the steady-state sensitivity is a local sensitivity measurement that perturbs one parameter and fixes the other parameters. As shown in Figure 9, we calculated the NSSS of all states with respect to the output \( m_1 \) for both constant control and the feedback control algorithm. The NSSS of the feedback control case has significantly smaller magnitude in terms of average maximum sensitivities for all states (0.93 v.s. 3.42) and output state (0.02 v.s. 1.66). Especially for the output state, the magnitude of NSSS was reduced 100-fold. This implies that the parameter uncertainty does not affect the steady state of the output. The feedback control law (81) can always drive the output to the reference level subject to uncertainties and perturbation in the parameters locally.

Biologically, we can conclude from the above data that the feedback control is less influenced by disturbances within the system, as shown in Figure 9. For mRNA expression, this means that a feedback control prevents the system from being overly sensitive to any specific parameter. The perturbations or parameter uncertainty in any parameter will not cause a significant deviation to the steady mRNA level. This is necessary for complex biological systems, where parameter uncertainty and perturbations widely exist. If the system was overly sensitive over some parameters, then it would be difficult to maintain the mRNA at a desired level. A series of checks and balances are always present to prevent the system from fluctuating out of control.

Discussion

This paper employs control theory and a systems engineering approach to demonstrate the regulation in silico of the budding

![Figure 6. Comparison between constant and feedback control for the Reduced Order Model.](https://www.plosone.org/doi/10.1371/journal.pone.0019353.g006)
yeast, *Saccharomyces cerevisiae*, into a desired gene expression and/or metabolic activity pattern. The GAL network is used as an example to demonstrate the effectiveness of a systems approach for complex cellular system control. We demonstrate that although the structure and computational models are complicated, it is possible to use a “simple” control algorithm to manipulate the system. According to the local parameter sensitivity analysis, some terms can be eliminated from the model without adversely affecting its long-term behavior, and the control design based on the reduced model can still be applied to the original model. Both constant control and feedback control laws have been designed for the galactose network. To evaluate the control performance, we conducted local steady-state sensitivity and global sensitivity analysis. The results accordingly imply that feedback control significantly suppresses the parameter uncertainties. This sheds light on the potential to provide novel control components to eukaryotic transcriptional regulation networks, as well as other gene regulatory networks.

The approach we propose can not only be used to control the yeast GAL network, but the fundamental principles can be applied to a wide range of biological network control. Complex networks involving cell-cycle controls, DNA repair, and other genes of interest can be controlled in a similar manner, given an effective means of monitoring the output of the system. The most attractive application is the control of bioreactors. If done properly, the ability to yield a large amount of the target in a relatively short period of time, while minimizing the effects of toxic levels of gene expression products can be achieved. If these systems are tightly controlled at the cellular level, proteins of interest, such as immunogenic proteins used in the production of vaccines, restriction enzymes, biopharmaceuticals, biochemicals can be tightly regulated. This would allow for a cost-effective means of producing a large variety of products for research and commercial use. Applications to other systems include bacterial expression systems and even mammalian cell systems, based on the effectiveness of the bioreactor and the access to the requisite control variables. Of course, other physical and chemical parameters would be necessary to completely control a bioreactor-based system, including pH, temperature, and nutrient conditions. The major difference between these and the above approach is that the proposed approach focuses on cellular system control, which is still a widely open field. Our future research will implement this control system with a fully established microfluidic device to monitor the real-time expression of GAL-GFP fusion proteins. By validating the above control in an experimental environment we will demonstrate the usefulness and validity of our approach.

**Figure 7.** Effect of protein concentration on mRNA expression by feedback control on the GAL network. All states (unit: molec./cell) converge to a stable steady state, while $g_9$, $g_{10}$ and $g_{80}$ have slower convergence rates than the other states. The transient process of states $g_{80}$ and $g_{10}$ is listed in the small windows above. The overshoot of the states is kept in a reasonable range. No severe fluctuations or oscillations are found in the GAL network under feedback control.

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Figure 8. Comparison of referenced tracking between the original model and the reduced model using feedback control. Under the feedback control, the tracking performance of the original model and the reduced model is similar. Thus, the control design based on the reduced model can be applied to the original model directly.

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Figure 9. Comparison of normalized steady-state sensitivities (NSSS) between constant and feedback control. The NSSS of the feedback control case has significantly smaller magnitude in terms of average maximal sensitivities for all states (0.93 v.s. 3.42) and the output state (0.02 v.s. 1.66). The bars describe the maximum and minimum values of NSSS of the states with respect to each parameter. The solid lines denote the output NSSS on each parameter.

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Author Contributions
Conceived and designed the experiments: RY SL JW MJZ. Performed the experiments: RY SL JW MJZ. Analyzed the data: RY SL JW MJZ. Contributed reagents/materials/analysis tools: RY SL JW MJZ. Wrote the paper: RY SL JW MJZ.

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