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Protein-Level Control of Metabolism: Design Principles and Prospects from a Representative System

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Abstract: Significant evidence suggests protein-level or metabolic control is widespread and important in metabolic networks. However, the biophysical interactions responsible for flux control at the metabolic level are not nearly as well-characterized as those which are responsible for control at other biological levels, such as transcriptional regulation. This knowledge gap is a limiting factor in the application of engineered protein-level regulation in Metabolic Engineering for the rational and sensitive control of pathway flux. Here we apply an *in silico* dynamic numerical optimization approach to a representative branched pathway to understand how engineered allosteric regulation could be used to control flux. We consider inhibition sensitivity as a hypothetical tunable parameter to demonstrate that integration of allosteric and transcriptional regulation is necessary to stably achieve arbitrary targets for both downstream metabolite concentrations. We further show that the steady-state ratio of these metabolites can be controlled by tuning the sensitivity of allostery at the branch point. Finally, we demonstrate that system dynamics dictate which type of engineered control is optimal. This work has implications for the co-optimization of transcriptional and allosteric regulatory systems in metabolic networks and provides a framework for the design of allosteric regulation in engineered metabolisms.

Keywords: metabolic engineering, protein engineering, regulation, metabolism, allosteric regulation, control

1. INTRODUCTION

Metabolic Engineering seeks to replace traditional means of chemical production with microbial chemical factories through genome modelling and manipulation. Typically, this is achieved through the deletion of metabolic pathways and/or insertion of heterologous pathway genes to direct metabolic flux toward valuable native and non-native products (Woolston, *et al* 2013). A wide range of optimization techniques at various scales have been explored by metabolic engineers. This study is concerned with enabling tools dynamic engineering of flux. Typically dynamic control involves engineered regulation of flux through the creation of sensor-reporter gene networks which modulate expression of a target enzyme as a function of the intracellular concentration of a downstream metabolite or exogenous chemical (Venayak, *et al* 2015). The widespread use of such gene networks has been facilitated by the rapid growth of Synthetic Biology and the concomitant explosion in the tools available for the efficient engineering of genetic systems.

The field of Protein Engineering has seen similar, though necessarily slower advances in recent years. Protein design, unlike gene network design, is limited largely by the computational power available to engineers (Marcheschi, *et al* 2013). Despite this, *de novo* engineered proteins have been applied in at least two significant Metabolic Engineering projects (Leonard, *et al* 2010) (Siegel, *et al* 2015). Additionally, several methods for the introduction of allosteric control of activity into unregulated proteins have been established and validated (Ostermeier, 2005) (Taylor, *et al* 2016). These techniques have primarily focused on furthering the structural and evolutionary understanding of the phenomenon of allostery (Lindsley and Rutter, 2007), but we suggest that they could be applied in the dynamic control of metabolism analogously to gene networks. In this scenario, an engineered enzyme would act as both sensor and regulator to control metabolic flux.

A significant and growing body of work has characterized the role of metabolic-level control of flux for various network topologies. Metabolic control analysis (MCA) in particular has been useful to demonstrate the significance of metabolic control in regulating pathway flux (Wang, *et al* 2004) (Fell and Sauro, 1985). Other modelling techniques have been used to demonstrate both the theoretical interactions of metabolic and transcriptional control (Oyarzun, *et al* 2007) and to recapitulate these effects as observed in a model biological system (Zaslaver, *et al* 2004). From this work, it is clear that metabolic changes are significant drivers of flux changes (Gerosa, *et al* 2015) and therefore that manipulation at the metabolic-level could be used to optimize engineered metabolisms.

However, the application of these conclusions to the rational design of metabolic control is relatively difficult within these

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modelling frameworks, as they describe potentially tunable biophysical interactions in abstract mathematical terms only. For example, feedback inhibition of enzyme activity is characterized via elasticities in MCA (Fell and Sauro 1985), which are related to, but have no concrete connection to effector binding strength and/or mode of action – i.e. enzyme parameters which are rapidly becoming tunable as Protein Engineering matures. Additionally, many of these approaches rely on linearization for convenience, thereby ignoring the inherently and potentially useful non-linear nature of enzyme inhibition (Wang, *et al* 2004).

We therefore build from this body of work by numerically simulating a branched pathway with explicitly-defined, non-linear, effector-enzyme relationships. Our intention with this approach is to directly link a tunable, biophysical interaction with the flux effects of such an interaction so that it can be optimized. We selected a branching pathway because this is highly relevant for Metabolic Engineering, which is concerned with diverting flux at target nodes toward economically productive pathways. In this topology, metabolic control interactions are most efficient at the entry points to either downstream metabolic branch (Savageau, 1974), so we place the target engineered inhibition at the entry point to one of the branches. We begin by optimizing the global transcriptional control of our system under different sensitivities of allosteric regulation to show that allostery is required for optimal partitioning of flux among the downstream branches to reach relatively arbitrary targets for both terminal metabolites. Then we simulate the system under three different control regimes—transcriptional only, allosteric only, and integrated—to demonstrate that allosteric control of enzyme activity alone can effectively partition flux among the downstream branches, but that the optimal control regime depends strongly on system dynamics and the target steady-state ratio of terminal metabolites in the system.

2. SYSTEM DESCRIPTION

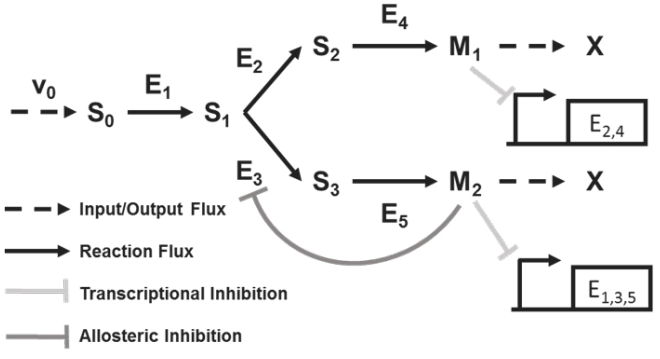

Fig 1. Model system. S_0 enters the system at constant flux v_0 and is converted into either terminal metabolite M_1 or M_2 in a step-wise fashion via the action of enzymes E_i. Terminal metabolites leave the system at rates proportional to their concentrations. Regulatory interactions include transcriptional feedback inhibition of enzymes 1, 3, and 5 by metabolite M_2, and enzymes 2 and 4 by metabolite M_1. In scenarios in which allosteric inhibition is considered, M_2 additionally inhibits the activity of E_3 directly.

In our system (Fig. 1), substrate S_0 enters at a constant flux v_0. It is converted to metabolite S_1 which can enter either of two branching pathways with terminal metabolites M_1 and M_2. Five enzymes (E_i) catalyse the conversion of S_0 to either terminal metabolite. With the exception of E_3, their activity was modelled using standard Michaelis-Menten kinetics. E_3 activity was modelled with Michaelis-Menten kinetics with uncompetitive inhibition. The terminal metabolites leave the system at a rate proportional to their concentrations and enzyme expression is controlled by the concentration of either terminal metabolite in a dose-responsive way.

The kinetic equations describing this system are as follows:

$$\frac{dS_0}{dt} = v_0 - \frac{k_{cat}E_1S_0}{K_m + S_0} \tag{1}$$

$$\frac{dS_1}{dt} = \frac{k_{cat}E_1S_0}{K_m + S_0} - \frac{k_{cat}E_2S_1}{K_m + S_1} - \frac{1}{\alpha}\frac{k_{cat}E_3S_1}{K_m + S_1} \tag{2}$$

$$\frac{dS_2}{dt} = \frac{k_{cat}E_2S_1}{K_m + S_1} - \frac{k_{cat}E_4S_2}{K_m + S_2} \tag{3}$$

$$\frac{dS_3}{dt} = \frac{1}{\alpha}\frac{k_{cat}E_3S_1}{K_m + S_1} - \frac{k_{cat}E_5S_3}{K_m + S_3} \tag{4}$$

$$\frac{dM_1}{dt} = \frac{k_{cat}E_4S_2}{K_m + S_2} - \gamma M_1 \tag{5}$$

$$\frac{dM_2}{dt} = \frac{k_{cat}E_5S_3}{K_m + S_3} - \gamma M_2 \tag{6}$$

$$\frac{dE_i}{dt} = \frac{\beta_i}{1 + \frac{R}{k_i}} - \gamma E_i \tag{7}$$

Where R is the fraction of repressor protein (total concentration assumed constant) bound to the control signal:

$$R = \frac{M_j}{1 \cdot 10^{-4}\, mM + M_j} \tag{8}$$

In (8) j = 1 for i = 2, 4 and j = 2 for i = 1, 3, 5.

Here γ is the rate constant for enzyme degradation and terminal metabolite flux out of the system. We describe it as the "turnover rate constant". The use of first-order kinetics for both processes assumes that they are associated with growth and therefore that cell division is the dominant process by which both enzymes and the terminal metabolites leave the system. This allows for the examination of control dynamics relative to growth rate, as in Fig 5.

Uncompetitive allosteric inhibition ("allostery") in (2) and (4) is described by

$$\alpha = 1 + \frac{M_2}{K_I} \tag{9}$$

This assumes that the fraction of inhibited enzyme reaches equilibrium instantaneously relative to the timescale of transcriptional repression. In (9), K_I is the inhibition constant and it is inversely proportional to the sensitivity of inhibition.