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Robustness analysis of combined transcriptional and translational resource allocation controllers ⋆

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Abstract: Recent work on engineering synthetic cellular circuitry has shown that non-regulatory interactions brought about through competition for shared gene expression resources, such as RNA polymerase and ribosomes, can result in degraded performance or even circuit failure. Transcriptional and translational resource allocation controllers based on orthogonal 'circuit-specific' gene expression machineries have been separately designed to enforce modularity and improve circuit performance. However, combining these controllers can result in instability. Here we apply tools from robust control theory to study the impact of uncertainty due to experimental implementations on the operation of dual transcriptional-translational resource allocation controllers.

Keywords: Robustness, Synthetic Biology, μ-Analysis, Nonlinear Systems, Uncertain Systems

1. INTRODUCTION

Systems and control theory is now widely used in the field of synthetic biology to guide the design of novel genetic circuits. These synthetic circuits can be used to integrate external stimuli and guide microbial behaviour with numerous potential applications in a range of sectors. However, at present circuits often fail when previously characterised modules are implemented in different contexts (whether that is expression with different modules or in different strains). A key cause of context-dependent failure of gene circuits is the competition between different modules for scarce gene expression resources such as RNA polymerases and ribosomes (e.g. Gyorgy et al. (2015)). This means that apparently independent processes (e.g. Process 1 and 2 in Fig. 1) are linked through their resource use; as Process 1 utilises resources through k_1, a disturbance d_1 is applied to the resource pool which then impacts the value of k_2 driving Process 2 (and *vice versa*). The use of orthogonal transcriptional and translational systems has been proposed to relieve such host-circuit interactions by providing a set of 'circuit-specific' gene expression resources (Liu et al. (2018)). Combined with feedback control systems such 'resource allocation controllers' can be used to maintain the available resource pool, hence rejecting the disturbances d_1 and d_2 and improving the modularity of the circuit processes (Fig. 1b).

2. DESIGN OF A DUAL RESOURCE ALLOCATION CONTROLLER

Here we consider the combination of the transcriptional controller developed by Kushwaha and Salis (2015) and the translational controller developed by Darlington et al. (2018) to develop a dual transcriptional-translation resource allocation control system. The first controller consists of an orthogonal RNA polymerase which transcribes its own negative regulator. The second controller consists of an RNA which creates orthogonal ribosomes. The RNA's expression is itself controlled by a repressive transcription factor which is translated by the orthognal ribosomes.

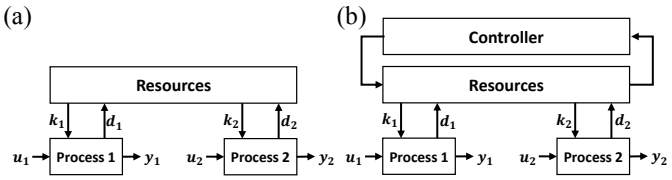

Fig. 1. (a) Open loop system. Both process 1 and 2 compete for the common pool of resources. Resources are utilised through k_i. Resource sequestration by the process i results in a retroactive disturbance to the resource pool through d_i. This results in coupling between the two processes as the magnitude of the net flux of resources to the Process i falls as k_i is impacted by all N distrubances d_j, for $j \in [1:N]$. (b) Closed loop system. The controller acts to reject the disturbances d_1 and d_2 hence maintaining k_1 and k_2.

We have previously demonstrated that combining the nominal (stable) controller designs developed by Kushwaha and Salis (2015) and Darlington et al. (2018) results in the emergence of instability when the dual controller system is applied to a simple two gene circuit *in silico* (Darlington and Bates (2019)). Here we consider different designs (i.e. different parameterisations) of the

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dual control system which govern the production rates of the orthogonal RNA polymerase and rRNA and the production rates and action of the two controller proteins. We identify two stable dual controllers which successfully decouple co-expressed genes at both the transcriptional and translational levels. Both controllers are able to reject disturbances to one gene (Process 1, shown in Fig. 2) as a second is induced (not shown). Note that controller 2 shows best nominal performance at the mRNA level by allowing for higher levels of production; this is likely to lead to reduced noise upon biological implementation.

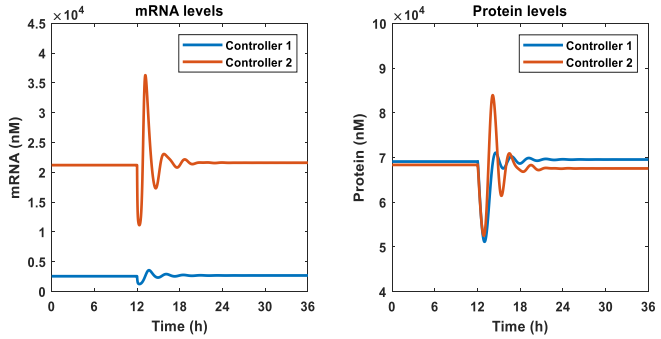

Fig. 2. Nominal performance of two alternative dual Tx-Tl control systems in response to the induction of a second gene at 12 h. This induction functions as a step disturbance applied to both the mRNA and protein of Process 1 (traces shown) as Process 2 utilises the same pools of RNA polymerases and ribosomes for its own expression.

3. ROBUSTNESS ANALYSIS

At present the design of genetic controllers is complicated by uncertainty in the kinetic parameters of biological 'parts'. Designs schemes such as those applied above may recommend a specific kinetic rate which is not yet available in a list of characterised 'parts'. Even where a desired part exists, context effects, e.g. the surounding DNA sequences or host strain genetic background, can cause subtle changes in binding rates (reviewed extensively in Cardinale and Arkin (2012)). To take account of these biological realities, here we assess the robustness of both controllers to parametric uncertainty; focusing on those parameters which can be experimentally manipulated and which govern the production rates of the orthogonal RNA polymerase and rRNA and the production rates and action of the two controller proteins.

The model of the control system is highly complex with multiple uncertainties that impact multiple species steady states. We apply the LFT-free μ analysis approach developed in Darlington et al. (2019) in order to rigorously quantify the robustness of the two controllers in Fig. 2. We first numerically solve the equations (as in Fig. 2) before linearising and calculating the uncertain Jacobian for the closed-loop system. We then apply the probabilistic algorithms in Darlington et al. (2019) to estimate the upper and lower bounds for μ across a range of frequencies (see Fig. 3). As shown in Fig. 3, our analysis indicates that the first controller is guaranteed to be stable for a parametric uncertainty level of up to 15%, since both μ-bounds are less than 1 at all frequencies. However, the second controller cannot be guaranteed to be stable for this level of uncertainty as the upper bound on μ is 1.65. This potential instability was confirmed via Monte Carlo simulations which identified a destabilising parameter combination within the allowable uncertainty range (Fig. 3, right inset).

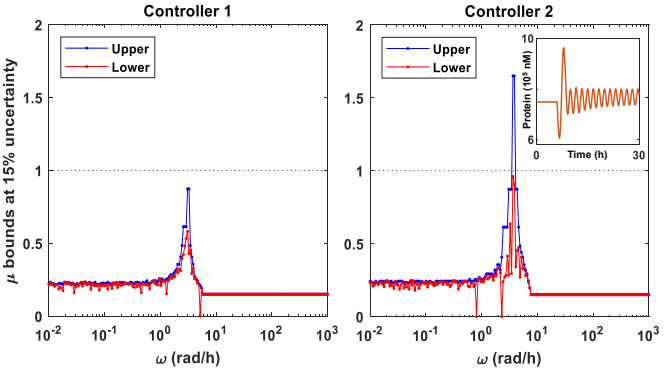

Fig. 3. μ analysis of the two dual controllers at 15% uncertainty. *Right inset*, destabilisation of controller 2 was confirmed via Monte Carlo sampling.

4. CONCLUSIONS

We have shown how the μ-analysis framework from robust control theory can be used to rigorously evaluate the relative robustness characteristics of different resource allocation controllers, in order to identify designs that are more likely to produce successful experimental implementations. Our analysis of two potential controllers revealed a classical performance/robustness trade-off, with the controller showing the best nominal performance exhibiting lower levels of robust stability.

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