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Contractivity of a genetic circuit with internal feedback and cell-to-cell communication ⋆

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Abstract: We consider a realistic model of the synthetic gene circuit combining cell-to-cell communication system via quorum sensing, and a synthetic repressible promoter implementing intracellular negative feedback control. The circuit has been shown to increase robustness with respect to both extrinsic and intrinsic noise elsewhere. As a first step towards an analytic analysis, in this paper we use contraction theory to perform a stability analysis. From it, we infer the components of the circuit most affecting the rate of contractivity, using biologically sensible values of the circuit parameters.

Keywords: Synthetic biology, Cell-to-cell communication, Differential stability, Contractivity

1. INTRODUCTION

Two key generic challenges of the bioprocess industries are developing efficient production systems for protein synthesis and expression, and the rational design and optimization of synthetic pathways for the synthesis of commodities. Heterologous protein synthesis starts by introducing an exogenous protein-coding gene in the cell, so that this produces the corresponding protein. Traditionally, for the design, optimization and control of bioprocesses, the population of microorganisms has been typically considered as an aggregate quantity characterized by averaged properties (Carlquist et al., 2012). Yet, it is a fact that even isogenic (i.e. with the same genetic content) microbial populations have certain degree of heterogeneity. Indeed, individual microorganisms, even if part of a clonal or isogenic population, may differ greatly in terms of genetic composition, physiology, biochemistry, or behavior (Elowitz et al., 2002; Toni and Tidor, 2013; Picó et al., 2015). In particular, the phenomenon of phenotypic noise is described as variation within an isogenic population due to fluctuations in gene expression of single cells (Toni and Tidor, 2013). This heterogeneity at the population level has been shown to be one of the causes of decrease in productivity when scaling-up to an industrial production bioprocess (Fernandes et al., 2011). Characterization and control of protein expression moments (mean and variance) across the cell population is, thus, a hot topic (Sánchez and Kondev, 2008; Weber and Buceta, 2011; Vignoni et al., 2013; Mélykúti et al., 2014; Oyarzu n et al., 2014) of relevance also for synthesis of commodities through synthetic pathways (Oyarzuun, 2011).

Dealing with the problems above requires both appropriate dynamic predictive models, and designing dynamic controls of the synthetic pathways and expression systems (Holtz and Keasling, 2010; Singh, 2011; Vignoni et al., 2013). An area of particular relevance is the design of collective cell behavior, whereby a prescribed population response results from the interaction between individual cells. A common approach to induce collective behavior is to use cell- to-cell communication mechanisms. These typically rely on the quorum sensing machinery from *V. fischeri*, and have been used for diverse purposes such as population synchronization (Nikolaev and Sontag, 2015), cell density-based control of gene expression (Williams et al., 2013), engineered cell social behavior Youk and Lim (2014), etc. The effect of cell-to-cell communication on noise regulation was also analyzed in (Tanouchi et al., 2008; Weber and Buceta, 2011; Boada et al., 2015). Following this line, in (Vignoni et al., 2013) we proposed a preliminary synthetic gene circuit aimed at controlling the mean and variance of protein expression across a cell population. The circuit uses both an intracellular negative feedback loop, and an external loop using quorum sensing as a means for cell-to-cell communication. Using a very simplified model we proved that the trajectories of the cell states converge to a closed region, wherein expressions for the mean and variance of the states could be obtained, using a linearised model, when variability in a key parameter of the circuit is present.

In this paper we consider a realistic model of the synthetic gene circuit. Analysis of stability of the circuit cannot be addressed using standard Lyapunov techniques, given the complexity of the model. Provided the system has a stable equilibrium point, a linearised model could be used to infer the effects of the circuit both on intrinsic and extrinsic noise, using an approach analogous to (Tanouchi et al., 2008; Vignoni et al., 2013). In this work, using contraction theory methods derived in (Russo and Di Bernardo, 2009; Russo et al., 2011; Margaliot et al., 2016), and realistic values for the parameters of the circuit, we derive the conditions under which the system has a stable equilibrium point.

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The rest of the paper is organized as follows. In Section 2 we describe the gene synthetic circuit, and its mathematical model. In section 3 we perform the contraction analysis and, in section 4, its results for realistic values for the parameters of the circuit are derived. Finally, some conclusions and future work are drawn in the last section.

2. SYSTEM DESCRIPTION

Description of the synthetic gene network.

The proposed gene synthetic circuit, depicted in Fig. 1, combines two engineered gene networks previously implemented in *E. coli*: i) a cell-to-cell communication system via quorum sensing (QS) (Fuqua et al., 2001) using the autoinducer molecule acyl homoserine lactone AHL, and ii) a synthetic repressible promoter (Egland and Greenberg, 2000; Vignoni et al., 2013) implementing a negative feedback control over the concentration of the protein LuxI, the synthase that produces AHL. The ultimate goal of the circuit is to control the expression of an heterologous protein of interest which could be encoded in the same coding sequence as LuxI. This way, control of LuxI will be tantamount to that of the protein of interest except for its translation step. Notice transcription has been identified to be the protein expression step most affecting variability (Guimaraes et al., 2014)

The internal feedback loop aims at reducing the variability of LuxI at each individual cell. It consists of the gene *luxR* constitutively producing the protein LuxR. On the other hand, the protein LuxI synthesizes the autoinducer of the AHL inside each cell. Then, AHL binds to LuxR creating the monomer $(\mathrm{LuxR}\cdot\mathrm{AHL})$. In turn, two molecules dimerize producing $(\mathrm{LuxR}\cdot\mathrm{AHL})_2$. This complex is a transcription factor for the synthetic repressible promoter $\mathrm{P_{luxR}}$ promoter, controlling the expression of LuxI. Thus, the dimer $(\mathrm{LuxR}\cdot\mathrm{AHL})_2$ inhibits the transcription of the gene *luxI* downstream the $\mathrm{P_{luxR}}$ promoter. Hence, the circuit internal loop has a negative feedback loop between the intracellular AHL and LuxI.

The outer feedback loop accounts for the passive diffusion of AHL across the cell membrane to the culture medium, thus acting as communication signal within the population. This signal can be used to induce coordination in the cell population.

For the circuit above, we consider the main biochemical reactions involved: the genes regulated transcription and translation processes, the hetero- and homodimerization reactions involving the inducer, and diffusion of the inducer through the cell membrane. We simplify transcription of genes *luxI* and *luxR* by considering k_{mLuxI} and k_{mLuxR} as the effective irreversible transcription rates. Besides, α_{mLuxI} represents the basal transcription of the repressor $\mathrm{P_{luxR}}$. We also model translation as an irreversible reaction with an average transcription rate accounting for the fact that binding of ribosomes to the ribosome binding site (RBS) is indeed reversible, and several ribosomes may translate a single mRNA molecule simultaneously. Monomerization and dimerization are considered as reversible reactions. The diffusion process of the inducer across the cell membrane is modeled as a pseudo-reaction, where $\mathrm{V_c} = \mathrm{V_{cell}}/\mathrm{V_{ext}}$ is the ratio between the cell and the culture volume, that allows to quantify the number of intracellular AHL or extracellular $\mathrm{AHL_{ext}}$ molecules. With these simplifying assumptions in mind, the resulting set of reactions is shown in

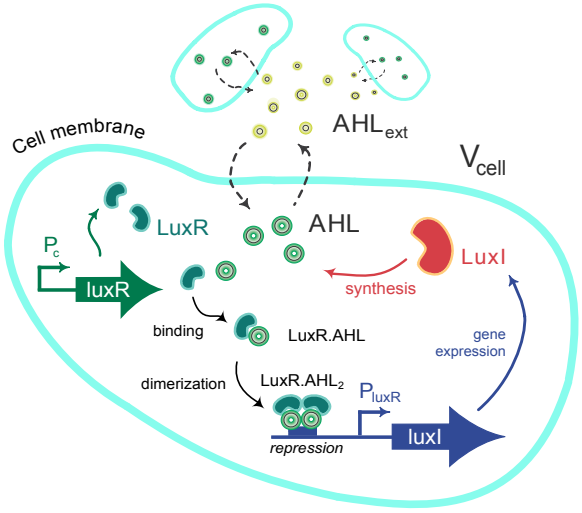

Fig. 1. Synthetic gene circuit.

(1). For convenience, we denote by DNA the free promoter of the gene *luxI*. The messenger RNAs of genes *luxI* and *luxR* are denoted as $\mathrm{mRNA_{luxI}}$ and $\mathrm{mRNA_{luxR}}$ respectively.

$$
\begin{aligned}
&\mathrm{DNA} \xrightarrow{k_{\mathrm{mLuxI}}} \mathrm{DNA} + \mathrm{mRNA_{luxI}}\\
&\xrightarrow{k_{\mathrm{mLuxR}}} \mathrm{mRNA_{luxR}}\\
&\mathrm{DNA(LuxR\cdot AHL)_2} \xrightarrow{\alpha_{\mathrm{mLuxI}} k_{\mathrm{mLuxI}}} \mathrm{DNA(LuxR\cdot AHL)_2} + \mathrm{mRNA_{luxI}}\\
&\mathrm{mRNA_{luxI}} \xrightarrow{k_{\mathrm{LuxI}}} \mathrm{mRNA_{luxI}} + \mathrm{LuxI}\\
&\mathrm{mRNA_{luxR}} \xrightarrow{k_{\mathrm{LuxR}}} \mathrm{mRNA_{luxR}} + \mathrm{LuxR}\\
&\mathrm{LuxI} \xrightarrow{k_A} \mathrm{AHL} + \mathrm{LuxI}\\
&\mathrm{LuxR} + \mathrm{AHL} \underset{k_1^-}{\overset{k_1^-/k_{d1}}{\rightleftarrows}} \mathrm{LuxR\cdot AHL}\\
&2(\mathrm{LuxR\cdot AHL}) \underset{k_2^-}{\overset{k_2^-/k_{d2}}{\rightleftarrows}} (\mathrm{LuxR\cdot AHL})_2\\
&(\mathrm{LuxR\cdot AHL})_2 + \mathrm{DNA} \underset{k_{lux}^-}{\overset{k_{lux}^-/k_{dlux}}{\rightleftarrows}} \mathrm{DNA(LuxR\cdot AHL)_2}\\
&\mathrm{AHL} \underset{\mathrm{DV_c}}{\overset{\mathrm{D}}{\rightleftarrows}} \mathrm{AHL_{ext}}\\
&\mathrm{AHL} \xrightarrow{d_A} \emptyset\\
&\mathrm{AHL_{ext}} \xrightarrow{d_{A_e}} \emptyset\\
&\mathrm{mRNA_{luxI}} \xrightarrow{dm_I} \emptyset\\
&\mathrm{mRNA_{luxR}} \xrightarrow{dm_R} \emptyset\\
&\mathrm{LuxI} \xrightarrow{d_I} \emptyset\\
&\mathrm{LuxR} \xrightarrow{d_R} \emptyset\\
&\mathrm{LuxR\cdot AHL} \xrightarrow{d_{RA}} \emptyset\\
&(\mathrm{LuxR\cdot AHL})_2 \xrightarrow{d_{RA_2}} \emptyset
\end{aligned}
\tag{1}
$$

Mathematical model.

The dynamical deterministic model corresponding to the biochemical reactions (1) can be obtained using the mass-action kinetics formalism (Chellaboina et al., 2009; Picó et al., 2015). Due to the constitutive expression of gene *luxR*, we can assume