

Tradeoffs in gene regulatory networks: the plasmid replication control problem

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Abstract—It has recently been demonstrated within the mathematical biology community how linear noise approximations can be used to analyze intrinsic fluctuations in biological systems. By interpreting the fluctuation dissipation theorem in a noise filtering setting, we illustrate in the paper ways of exploiting conventional tools from robust control theory to reveal tradeoffs in gene regulatory networks. The control of plasmid copy numbers in bacteria is discussed.

Index Terms—biological systems, robust control, gene regulatory networks

I. INTRODUCTION

The importance of intrinsic noise in gene regulatory networks has recently been emphasized with systematic analysis methods being proposed (see [1] and references therein). The 'stochastic chemical kinetics' that arise due to random births and deaths of individual molecules give rise to jump markov processes, often modelled by means of master equations and simulated with stochastic simulation algorithms [2]. This kind of intrinsic fluctuation is undoubtedly a major factor that needs to be taken into account when one attempts to pose certain criteria that would characterize the performance of a regulatory network under study: large variability is associated with poor regulation performance.

Nevertheless, if one tries to adopt a control theoretic approach in order to justify a control strategy for a particular regulation problem in a biological network, this intrinsic stochasticity turns out to introduce a major complication in the mathematical analysis. This is because the feedback mechanism itself will have to be a jump markov process since it will correspond to a number of chemical reactions.

Finding ways to analyze this kind of intrinsic noise is a problem that has received considerable attention by physicists from an early stage. Those go back to Einstein's relations indicating the correspondence of the damping coefficient in a fluid with the mean square of the fluctuations. This is a special case of the fluctuation-dissipation theorem which relates macroscopic parameters with intrinsic variability. An exact manifestation can be given for an Ornstein-Uhlenbeck process where it is known that the probability density function satisfies a linear Fokker-Planck equation. In this case the diffusion and drift coefficients are related, by means of a Lyapunov equation, to the steady state covariance matrix.

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In the paper we formulate the fluctuation dissipation theorem in a robust control setting using a linear filtering approach. The idea behind this is that by means of Van Kampens Ω expansion [3] one can justify a linear noise approximation for the intrinsic noise, which can be then analyzed by means of linear Fokker-Planck equations. Hence, in this way, conventional tools from control theory, such as the bode integral formulae and norm type cost functions to quantify robustness and performance, can be used to reveal tradeoffs in biological networks. In fact, we illustrate in an example of plasmid replication control in bacteria, that the *in vivo* reaction rates for the feedback mechanism are reasonable for the purpose of minimizing plasmid variability in the face of the transcription and replication delays inevitably present.

The paper is structured as follows. We give in section II a description of the underlying theory and indicate how a noise filtering setup can become relevant. This setting is used in the next section as a basis for analysis of the problem of replication control of a molecular species in a cell. The tradeoffs deduced are finally compared with *in vivo* values of the reaction rates of the mechanisms responsible for plasmid copy number control in bacteria.

II. THEORY

We describe in this section the linear noise approximation for the analysis of jump markov processes as this is given in [3]. It is then illustrated how, in such an approximation, the variance of the different species corresponds to the 2-norm of appropriate linear operators.

Using the notation in [4], we consider a biochemical system with N molecular species, R elementary reactions in some volume Ω . The number of molecules are denoted $X = [X_1, X_2, \dots, X_N]$ and v_{ij} are the number of molecules by which species j changes when an elementary reaction of type i occurs. Each reaction is assigned an intensity (or reaction rate) \tilde{f}_i i.e. reaction i occurs in a short time δt with a probability $\Omega \tilde{f}_i(\frac{X}{\Omega}, \Omega) \delta t$. The master equation for the probability $P(X, t)$ of having X molecules at time t is

$$\frac{dP(X, t)}{dt} = \Omega \sum_{i=1}^R \left(\prod_{j=1}^N E_j^{-v_{ij}} - 1 \right) \tilde{f}_i(X \Omega^{-1}, \Omega) P(X, t) \quad (1)$$

where the step operator E is defined to satisfy

$$E_j^{v_{ij}} g(\dots, X_j, \dots) = g(\dots, X_j + v_{ij}, \dots)$$

The linear noise approximation is obtained by decomposing X as

$$X = \Omega\psi + \Omega^{1/2}x \quad (2)$$

where $\psi = [\psi_1, \psi_2, \dots, \psi_N]$, $x = [x_1, x_2, \dots, x_N]$ and ψ satisfies the deterministic equations

$$\frac{d\psi_j}{dt} = \sum_i^N v_{ij} f_i(\psi) \quad (3)$$

where $f_i(\psi) = \lim_{\Omega \rightarrow \infty} \tilde{f}_i(\psi, \Omega)$. Note that ψ corresponds to the molecular concentrations at high volumes and let $\bar{\psi}$ be the steady state solution of (3) which is assumed to be unique. A second order expansion of (1) about $\bar{\psi}$ leads to a linear Fokker-Planck equation for the joint probability distribution $\Pi(x, t)$ of x :

$$\frac{\partial \Pi(x, t)}{\partial t} = - \sum_{j,k} a_{jk} \frac{\partial (x_k \Pi)}{\partial x_j} + \frac{1}{2} \sum_{j,k} b_{jk} \frac{\partial^2 \Pi}{\partial x_j \partial x_k} \quad (4)$$

$A = [a_{jk}]$ is the Jacobian for (3) about $\bar{\psi}$ i.e.

$$a_{jk} = \sum_{i=1}^R v_{ij} \left(\frac{\partial f_i}{\partial \psi_k} \right)_{\bar{\psi}}$$

and will be referred to as the drift matrix. $B = [b_{ij}]$ will be referred to as the diffusion matrix and is given by

$$b_{jk} = \sum_{i=1}^R f_i v_{ij} v_{ik} \quad (5)$$

The validity of the macroscopic law (3) has been shown in [5] in the sense that over finite times the trajectories $X(t)/\Omega$ of the jump markov process converge in probability to those of the macroscopic law as $\Omega \rightarrow \infty$. Nevertheless, even though the linear noise approximation can be very convenient in the derivation of analytical results it has certain limitations. For example, an infinite variance will be predicted for unstable macroscopic laws even though these could lead to a limit cycle behaviour. Also in the case of multiple equilibria transitions between these equilibria cannot be taken into account. It still is, however, a good approximation for local analysis about a particular equilibrium point in cases the fluctuations are small relative to the number of species present, and provides a good basis for getting an insight into how various mechanisms interact with one another.

It is well known [3] that the Fokker-Planck equation in (4) is satisfied by the probability density function of a random process described by a linear Langevin equation, i.e. a linear time invariant system with additive white gaussian noise. For example, in the case that each reaction affects only one species (i.e. $b_{ij} = 0$ for $i \neq j$) then $x(t)$ satisfies the Langevin equation

$$\dot{x}_j = \sum_k a_{jk} x_k + \sqrt{b_{jj}} n_j(t)$$

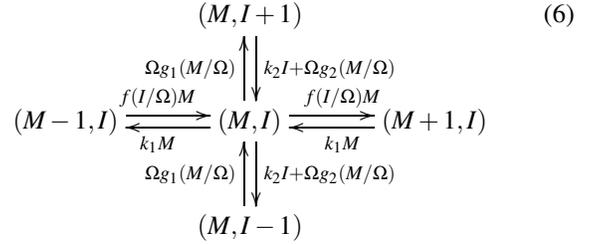
where $n_j(t)$ is the Langevin force that satisfies $\mathbb{E}(n_j(t)n_j(t')) = \delta(t-t')$. This formulation of a deterministic

system plus noise means that classical tools from robust control theory can now be used. For example the bode integral formula can help to reveal various tradeoffs in a feedback configuration. In addition, by considering the corresponding power spectra, it is easy to see that $\|T_{n \rightarrow x_j}\|_2^2 = \text{Var}(x_j)$ where $n = [n_1, \dots, n_j, \dots]$ is the vector of additive noise. This means one can also introduce delays in the feedback loop which can easily be dealt with in a linear system by means of frequency response methods.

III. REPLICATION CONTROL OF A MOLECULAR SPECIES

We analyze in this section a realistic scenario of replication control of a molecular species. This corresponds to a mechanism found in bacteria for plasmid copy number control. *In vivo* reaction rates for plasmid ColE1 are considered in the next section.

We consider a molecular species (we call it $M1$) in a cell that replicates and degrades spontaneously with certain rates. If these are equal and fixed the number of molecules would demonstrate a random walk with no steady state distribution being reached. Therefore some kind of feedback is needed to maintain a constant concentration. This is typically achieved by an extra species called the inhibitor molecule that affects the replication rate of $M1$. The inhibition mechanism is illustrated in the transition diagram below.



M is the number of molecules of the species we want to regulate, I the number of inhibitors and their densities are denoted as \hat{M} and \hat{I} respectively. The M molecules replicate with a rate $f(I/\Omega)M$ and decay with a rate $k_1 M$. Similarly inhibitors are generated by $M1$ with rate $\Omega g_1(M/\Omega)$ and spontaneously degrade with rate $k_2 I$. The term $\Omega g_2(M/\Omega)$ refers to possible degradation due to interaction with the $M1$ molecules as part of the inhibition mechanism. Note that the degradation rates k_1, k_2 also include terms corresponding to the cell volume growth so as to carry out the analysis in constant volume. Even though this growth might be deterministic we could incorporate it in the master equation by considering arbitrarily small increments with arbitrarily large reaction rates. If for example the volume is growing exponentially $V = V_0 e^{k_h t}$ a decrease term $-k_h \hat{M}$ will appear in the macroscopic law for the density as given by (3). This means that in the subsequent analysis it will appear in the master equation as a separate reaction with intensity $f_i = k_h$ and $v_{ij} = 1$. Nevertheless scaling f_i to $f_i K$ and v_{ij} to v_{ij}/K the corresponding term in the diffusion matrix $(f_i K) (\frac{v_{ij}}{K})^2$ tends to 0 as $K \rightarrow \infty$, even though the term $(f_i K) (\frac{v_{ij}}{K})$ remains invariant in the macroscopic law. Hence the volume growth

can be taken into account in the analysis without contributing directly in the intrinsic noise. For these reasons we denote \bar{k}_1 and \bar{k}_2 the decay rates k_1, k_2 respectively but without the volume increment terms. Let also \hat{I}_{ss} and \hat{M}_{ss} be the steady state values of the concentrations. By analogy with (3), the macroscopic equations for the system in (6) are

$$\begin{aligned}\dot{\hat{M}} &= f(\hat{I})\hat{M} - k_1\hat{M} \\ \dot{\hat{I}} &= g_1(\hat{M}) - k_2\hat{I} - g_2(\hat{M})\end{aligned}\quad (7)$$

Linearizing those about equilibrium we get

$$\begin{aligned}\frac{dm}{dt} &= \frac{\partial f(\hat{I}_{ss})}{\partial \hat{I}}\hat{M}_{ss}i \\ \frac{di}{dt} &= \frac{\partial g_1(\hat{M}_{ss})}{\partial \hat{M}}m - k_2i - \frac{\partial g_2(\hat{M}_{ss})}{\partial \hat{M}}m\end{aligned}$$

where $m = \hat{M} - \hat{M}_{ss}$, $i = \hat{I} - \hat{I}_{ss}$ and slightly abuse notation by writing $\frac{\partial f(\hat{I}_{ss})}{\partial \hat{I}}$ as $\frac{\partial f(\hat{I})}{\partial \hat{I}}$ evaluated at I_{ss} . Following the analysis in section II, the Jacobian is

$$A = \begin{bmatrix} 0 & \frac{\partial f(\hat{I}_{ss})}{\partial \hat{I}}\hat{M}_{ss} \\ \frac{\partial g_1(\hat{M}_{ss})}{\partial \hat{M}} - \frac{\partial g_2(\hat{M}_{ss})}{\partial \hat{M}} & -k_2 \end{bmatrix}$$

and by taking Laplace transforms we can also write the systems of equations as

$$\begin{aligned}\bar{m}(s) &= \frac{1}{s} \frac{\partial f(\hat{I}_{ss})}{\partial \hat{I}}\hat{M}_{ss}\bar{i}(s) \\ \bar{i}(s) &= \frac{1}{s+k_2} \left(\frac{\partial g_1(\hat{M}_{ss})}{\partial \hat{M}} - \frac{\partial g_2(\hat{M}_{ss})}{\partial \hat{M}} \right) \bar{m}(s)\end{aligned}$$

The reaction rates at equilibrium are

$$\begin{aligned}f_1 &= f(\hat{I}_{ss})\hat{M}_{ss} & v_{11} &= 1 \\ f_2 &= \bar{k}_1\hat{M}_{ss} & v_{21} &= -1 \\ f_3 &= g_1(\hat{M}_{ss}) & v_{32} &= 1 \\ f_4 &= g_2(\hat{M}_{ss}) & v_{42} &= -1 \\ f_5 &= \bar{k}_2\hat{I}_{ss} & v_{52} &= -1\end{aligned}$$

and the equilibrium conditions $f_1 = f_2$, $f_3 = f_4 + f_5$ also hold. Now $\hat{B} := B^{1/2}$ is therefore

$$\hat{B} = \begin{bmatrix} \sqrt{2f_2} & 0 \\ 0 & \sqrt{2f_3} \end{bmatrix}$$

The block diagram of the system is shown in figure 1. Note that we have also introduced a delay in the feedback loop corresponding to a replication delay T for the species M1. The variance of m is hence given by $\left\| T_{\begin{bmatrix} n_1 \\ n_2 \end{bmatrix} \rightarrow m} \right\|_2^2$ in the linear noise approximation. And since we are assuming a decomposition $M = \Omega\hat{M} + \Omega^{1/2}m$ as in (2), the actual variance of the molecules M about $\Omega\hat{M}_{ss}$ is $\Omega\text{Var}(m)$.

For convenience in the analysis presented in section IV-B we use the notation below.

$$\begin{aligned}K_I &= \frac{\partial f(\hat{I}_{ss})}{\partial \hat{I}}\hat{M}_{ss}, & K_M &= \left(\frac{\partial g_1(\hat{M}_{ss})}{\partial \hat{M}} - \frac{\partial g_2(\hat{M}_{ss})}{\partial \hat{M}} \right) \\ \eta_1 &= \hat{B}_{11}, & \eta_2 &= \hat{B}_{22}\end{aligned}$$

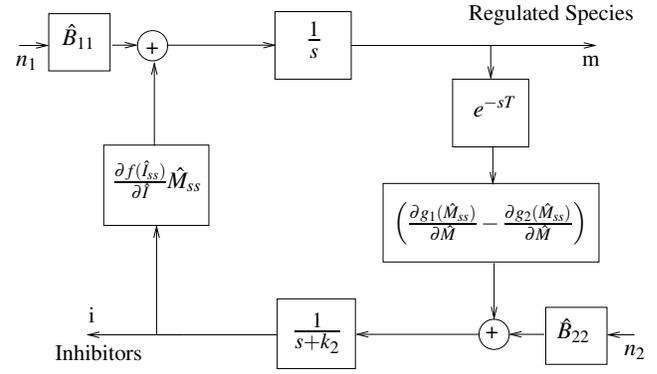


Fig. 1. Block diagram of an inhibition mechanism for regulation of a replicating molecular species.

Note that the norm is given by

$$\begin{aligned}\left\| T_{\begin{bmatrix} n_1 \\ n_2 \end{bmatrix} \rightarrow m} \right\|_2^2 &= \|T_{n_1 \rightarrow m}\|_2^2 + \|T_{n_2 \rightarrow m}\|_2^2 \\ &= \left\| \frac{(s+k_2)\eta_1}{s(s+k_2) - K_I K_M e^{-sT}} \right\|_2^2 + \left\| \frac{K_I \eta_2}{s(s+k_2) - K_I K_M e^{-sT}} \right\|_2^2\end{aligned}\quad (8)$$

IV. PLASMID COPY NUMBER CONTROL

A regulation mechanisms for plasmid copy number control in bacteria, is being analyzed in this section. By considering *in vivo* values for the reaction rates, the extent to which these mechanisms are behaving optimally with respect to minimizing variability in plasmid concentration is being investigated.

A. Background

1) *Bacteria*: Bacteria are simple, unicellular organisms. They use basic methods to reproduce and maintain genetic information (DNA), when compared to higher organisms such as animals. DNA in the form of the bacterial chromosome floats freely in the bacteria. This contains all the essential information that the bacteria need to manufacture the proteins necessary for existence. When the bacteria reproduce, another copy of the DNA is replicated, and the cell doubles in size, before splitting into two cells. Each cell contains one copy of the DNA and can go on to reproduce many times. For survival it is important that reproduction occurs quickly. It is also important that bacteria can respond quickly to changes in their surroundings by producing appropriate proteins. For example, in an environment containing a particular drug it is desirable to produce a protein that gives resistance to that drug.

2) *Plasmids*: Plasmids are small rings of DNA found outside of the chromosome in bacteria. Normally plasmids provide advantageous characteristics such as resistance to drugs, but can be modified so that bacteria produce useful products (they can be genetically engineered to form what is known as a vector). This is achieved by inserting genetic code for a gene of choice into cells. The process involves cutting the plasmid at an appropriate point, inserting DNA

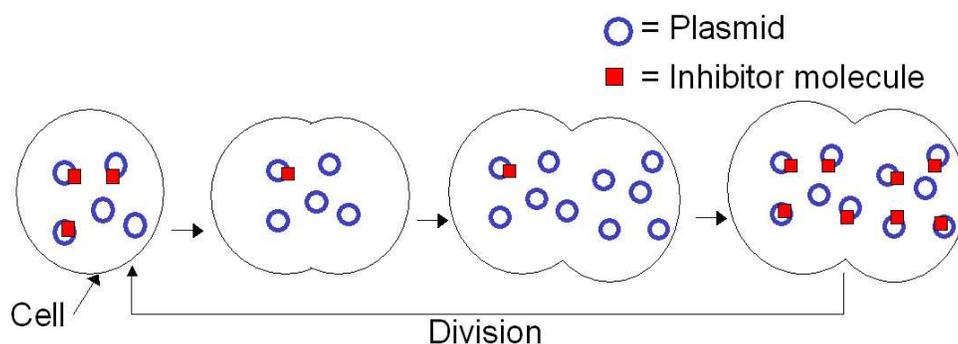


Fig. 2. The Plasmid-Inhibitor feedback loop

sequence of choice, and reattaching the ends to form a ring again. These can be drawn from a wide range of fields, for example therapeutics (e.g. insulin) and flavour enhancers (e.g. monosodium glutamate). The vectors are then inserted into a host cell such as *Escherichia coli*. The host cells are then cultured on a large scale, and will secrete the useful protein, which is extracted and purified.

3) *Plasmid copy number control*: Maintaining an appropriate number of plasmids (plasmid copy number) in a cell is important. Too many plasmids uses too many resources for their upkeep, so that the bacterium suffers a metabolic load and is unable to reproduce at a fast enough rate to compete with bacteria containing fewer plasmids. Too few plasmids mean that there is a greater chance that when the bacteria divide, some bacteria may not contain any plasmids. These bacteria would have a lower metabolic load, and could reproduce at a much higher rate. This would result in a population of bacteria with an overall decreasing number of plasmids, perhaps resulting in a plasmid being lost to the whole population. Therefore in nature there must exist mechanisms that ensure that the number of plasmids is kept at an ideal level. Of course, this is significant in industry too, as it is essential to maintain modified plasmids at a suitable copy number to avoid potential loss of the modified plasmid and considerable investment.

4) *Control mechanisms*: During reproduction, the bacterial chromosome is copied and moved to opposite ends of the cell before it divides (segregation). Bacteria have a mechanism to ensure that this always happens, called active partitioning. Plasmids have different mechanisms to control copy number (the number of plasmids in the cell) during and after segregation. The type of mechanism normally depends on the copy number of the plasmid, which in turn depends on the size of the plasmid. Low copy numbers (a few per cell) are associated with large plasmids, and higher copy numbers are linked to smaller plasmids. Larger plasmids require more resources for maintenance, and consequentially have smaller copy numbers.

As the cell volume doubles during a reproductive cycle, the number of plasmids must also double to keep the same concentration of plasmids after segregation. This is true regardless of the partitioning mechanism used during cell division. To ensure that copy number is maintained, lower

copy number plasmids use active partitioning. This can be further sub-divided into pair-site partitioning and equi-partitioning. Pair-site partitioning makes certain that one plasmid is guaranteed in each cell after segregation, with the other plasmids being randomly distributed. Equi-partitioning gives an equal distribution of plasmids between the cells.

Higher copy number plasmids rely on the low probability of one cell containing much fewer plasmids than the other after division. This is because there is equal probability of a plasmid being found in either of the cells after segregation, as Brownian motion will randomly distribute the plasmids. However, there will be inevitably some variation in the way in which the plasmids are divided between the two cells, due to the random nature of the division. Regulation mechanisms seek to reduce the effect of the variation, and return the plasmid concentration to the optimum level. We concentrate here on trying to improve the understanding of this high copy number mechanism. Improved understanding of the mechanism will be useful in industrial applications to allow optimal use of plasmids to produce useful proteins.

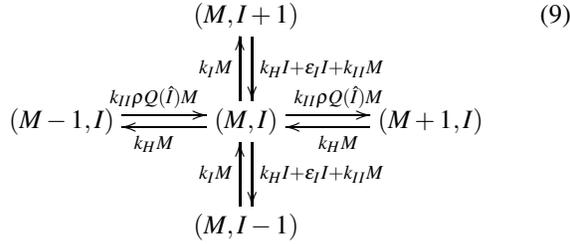
5) *Chemistry of the regulation mechanism*: The regulation mechanism for the plasmid ColE1 relies on negative feedback to maintain the optimal concentration of plasmids in a cell. This is achieved through the interaction of the plasmids and inhibitor molecules, known as RNA I. These are transcribed from the plasmid from the complementary strand of the coding region for RNA II, a primer for the plasmid replication. The inhibition takes place indirectly with RNA I forming a complex with RNA II within a certain inhibition window, i.e. part of the RNA II transcription process. However, inhibitors are unstable, and spontaneously decay with a time constant, allowing the plasmids to replicate. New inhibitors are formed more readily when more plasmids are present. Over time the number of plasmids will steadily increase in line with the cell volume, keeping plasmid concentration constant. The feedback is provided by the interaction between the inhibitors and the plasmids. Figure 2 shows this process.

As it has been emphasized in the introduction the challenge with such a feedback regulation mechanism lies in the fact there is intrinsic noise associated with both the plasmid and the inhibitor formation/degradation. It is therefore intriguing to contemplate whether evolution has led to a

behaviour that minimizes the noise, even in such a nontrivial setting.

B. Analysis

The copy number control problem for plasmid ColE1 has been extensively studied in [6], [7]. We concentrate here on the effect that delays can have in regulation performance. The transition diagram is given below (plasmid and inhibitor number are denoted by M, I respectively). As in section III we assume a constant cell volume by introducing a degradation rate associated with an exponential volume growth.



where the different rates/constants are:

k_{II} RNA II transcription rate

k_I RNA I transcription rate

ε_I spontaneous degradation rate for RNA I

k_H host cell growth rate

$Q(\hat{I})$ Probability that replication priming by RNA II is not inhibited by RNA I

ρ probability a mature primer initiates plasmid replication

The macroscopic equations for the densities are therefore

$$\begin{aligned}
 \dot{\hat{M}} &= k_{II} \rho Q(\hat{I}) \hat{M} - k_H \hat{M} \\
 \dot{\hat{I}} &= (k_I - k_{II}) \hat{M} - (k_H + \varepsilon_I) \hat{I}
 \end{aligned}$$

and the analysis in section III can directly be applied.

We now try to see the extent to which the plasmid variability is being minimized bearing in mind the presence of replication and transcription delays (we denote the total delay as T). We first evaluate the parameter $k_2 = \varepsilon_I + k_H$ and the delay T . It is known that the cell generation time (time it takes for cell volume to double) for bacterium *E. coli* under optimum growth conditions is 20 minutes. Thus $k_H = \ln(2)/1200$. Also from [6] (figure 5 in that paper which is based on work in [8]) $\varepsilon_I/k_H \approx 30$. Hence $\varepsilon_I + k_H \approx 0.018$. The plasmid replication time is estimated to be 26 seconds (20bp/s for first 400bp by means of pol I and 1000bp/s for the remaining 6-7kbp by means of pol III, see [9]). The RNA II transcription time is estimated to be 18s (550 bp at about 30bp/s). Therefore we assume a total delay in the feedback loop of 44s.

We next find the loopgain $K_I K_M$. Exponential inhibition is assumed (see [6],[7])

$$Q(\hat{I}) = e^{-\frac{\hat{I}}{C_I}}$$

where C_I is an inhibition constant. The gain $K_I K_M$ turns out to be at steady state

$$(k_H)^2 \left(1 + \frac{\varepsilon_I}{k_H}\right) \ln\left(\frac{k_H}{\rho k_{II}}\right)$$

From [7] $\frac{\rho k_{II}}{k_H}$ is estimated to be in the range [5, 100] (we use $\frac{\rho k_{II}}{k_H} \approx 50$) so $K_I K_M = 0.40 \times 10^{-4}$. Also using the fact that $K_M = k_I - k_{II}$ and the values $k_{II}/k_I \approx 0.3, \rho \approx 0.5$ ([7], [6]) the individual values of K_M, K_I are $K_M = 0.135, K_I = 3.0 \times 10^{-4}$.

Note first that $K_I \ll K_M$ so that the inhibitor intrinsic noise (second term in (8)) is not amplified round the loop. Figure 3 shows how the relative plasmid variability (as predicted from the linear noise approximation (8)) varies with increasing loopgain (the inhibitor noise term is assumed to be negligible as noted above). Note that the *in vivo* value of $K_I K_M$ is close to the one that achieves minimum variance. Also the minimum variance is only by a factor of 2 smaller than the one predicted with *in vivo* reaction rates.

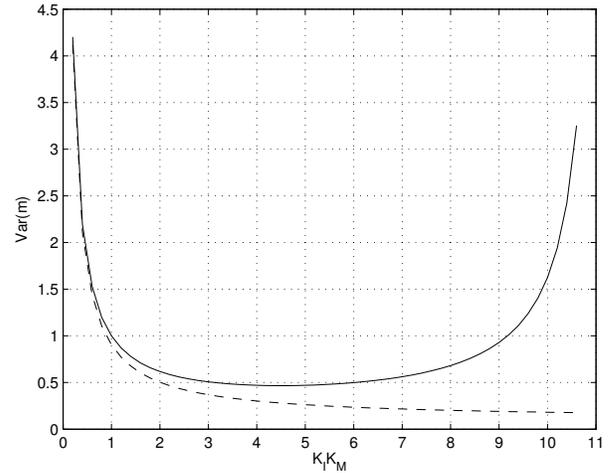


Fig. 3. Plasmid variance vs loopgain with these being normalized by corresponding *in vivo* values. The solid line corresponds to a delay of 44s and in the dashed line the delay is zero.

V. CONCLUSIONS

It has been shown in the paper how the fluctuation-dissipation theorem can be interpreted within a noise filtering framework such that one can use tools from robust control theory to analyze biological networks. Based on this setting the problem of regulating a replicating species in the presence of delays has been analyzed. By quantifying intrinsic noise as the 2-norm of a deterministic system optimal values have been conjectured for various reaction rates. These have been compared with *in vivo* values in *E. coli* for the mechanism of copy number control of plasmid ColE1 and a close match has been observed.

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