Proceedings of the 45th IEEE Conference on Decision & Control
Manchester Grand Hyatt Hotel
San Diego, CA, USA, December 13-15, 2006

On the modelling of a bistable genetic switch

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***Abstract*— A bistable switch is a common motif in a genetic regulatory network. There have been relatively few *in vivo* measurements made of such a network. Its natural closed loop nature makes the living switch difficult to measure experimentally. Hence not much have been reported on derivation of a model from *in vivo* data, which is expected to be different from the macroscopic scale *in vitro* measurements. We present a heuristic for modelling a naturally occurring switch from relatively few *in vivo* experimental data points, yielding a model suited to dynamical simulation, and give predictions of the unmeasured protein in the system.**

I. INTRODUCTION

A gene function can be conveniently represented as a (controlled) switch, where the ON state indicates expression of the gene and hence synthesis of gene product(s), and the OFF state otherwise. This representation makes a genetic switch one of the most common and versatile components in the gene-protein interaction network, representing cellular functions such as transcriptional control, "positive"/"negative" feedback[1], and building of cascading and toggle systems ([6], [22]).

A gene-protein interaction network is commonly thought to be one of the decoders that unlocks the genomic code into its phenomic manifestations; answering questions such as why is it that two individuals with very similar genomic sequence (or even identical) can look noticeably different or have different susceptibility to a given disease. But constructing models of such a network is no trivial exercise. Firstly, these networks are usually very large and complex. Furthermore, the development of techniques for estimating the rate parameters *in vivo* is still in their infancy. In its natural state, a gene regulatory network would be in closed–loop, so measurements made would not be indicative of the full operating range. Even when open–loop experiments can be devised, some proteins are simply hard to measure.

This paper illustrates a heuristic used to construct a model of a well known genetic switch, using few *in vivo* measured data points, qualitative knowledge of how the known components of the switch behave, and *in vitro* measurements as initial estimates.

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[1]the quotations is intended to stress that positive and negative feedback is terminology widely used in biological literature, which has a different meaning to that in control literature; this will be explained in the system described in this paper

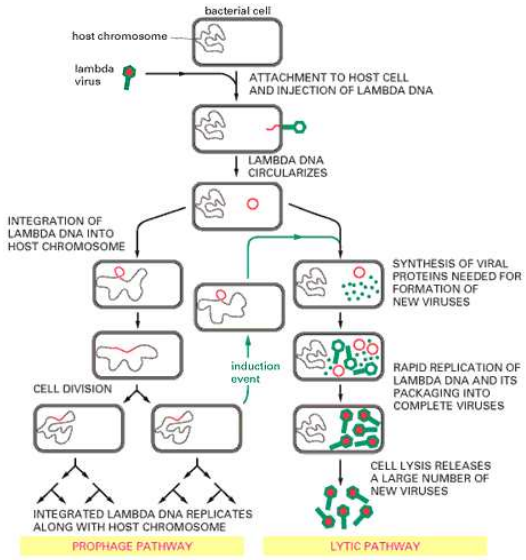

Fig. 1. The life cycle of bacteriophage-λ [11]

A. Genetic switching in bacteriophage-λ

A naturally occurring genetic switch can be found in the bacteriophage-λ, which infects the bacterium *Escherichia coli*. It is a two-state toggle switch, determining the life–cycle of the phage. A bacteriophage is a virus which infects (and consumes, hence the name) bacterial hosts, by inserting its DNA into the bacterial cell. It can enter a virulent phase, during which it reproduces and within a short amount of time lyses (destroys) the host. Some phages such as the λ-phage can enter a dormant stage, at which the phage DNA lysogenise (integrates) with the bacterial DNA and is copied with the host's during mitosis. The λ-phage does not kill the host but monitors its status. When the condition worsen, due to increase in temperature or depletion of nutrients, for example, the viral DNA excises itself from the host and goes into the lysis phase [3].

The life–path of the λ-phage is controlled by a bistable switch, comprising two mutually repressing proteins. The protein CI represses the virulent state and hence is also called λ repressor, and maintains lysogeny. The protein Cro plays a central role in excision of the λ-phage's DNA from the host's, construction and duplication of the phage components, and lysis of the host. Unless the host's condition is unfavourable, the λ-phage initially enters the lysogenic state upon infection. Lysis is induced in the presence of UV light, whereupon CI loses the ability to repress Cro, and the Cro-dominant state takes over [15].

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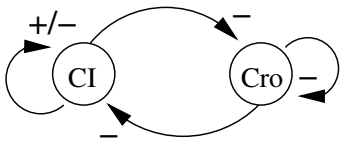
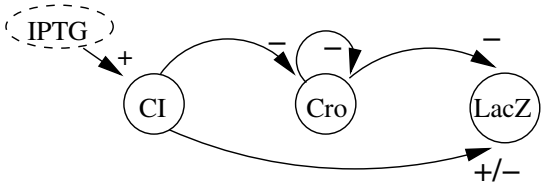


(a) Mutual repressor in λ phage



(b) Open loop experiment

Fig. 2. The regulating proteins in the λ-phage switch

These two proteins have the function of repressing each other, and above a threshold amount/concentration, start to repress their own synthesis (a phenomenon understood as auto-repression or auto-regulation). The CI protein also enhances its own synthesis at concentrations lower than the threshold, due to its structure and cooperative binding function [15]. In the biological literature, when a substance facilitates or enhances the expression of a gene (and hence the synthesis of its product), the relation is referred to as "positive feedback," and when it repressed the expression of a gene, the relation is called "negative feedback." These terms are very commonly used, and the difference in meaning should be noted. A gene/protein network is often represented by a directed graph, where the vertices represent the chemical species (genes or gene products, i.e. RNA or protein), and the arcs represent the relations; enhancing is indicated by a '+' sign and repressing by a '-', as depicted in Figure 2.

B. Problem description

Even though the λ-phage is a very well studied system, being the first known naturally–occurring genetic switch, there are aspects of the system that are yet to be discovered, such as a long-range cooperative binding between CI proteins [4]. Bacteriophage researchers are now turning their attention to functions of other proteins, such as Cro.

A major challenge in these studies arises in the form of the measurement of protein levels *in vivo*. Despite being well studied [20], [17], [23], [2], [21], the proteins have been measured *in vitro*, which brings questions on the accuracy of these results in much lower concentrations *in vivo*. Khammash reported the discrepancy between the results of the deterministic simulation and the mean of the stochastic simulation [10], due to a phenomenon called "stochastic focusing" [14]. The fact that this switch is in closed–loop introduces another challenge, as measurements are performed when the system is already in steady–state.

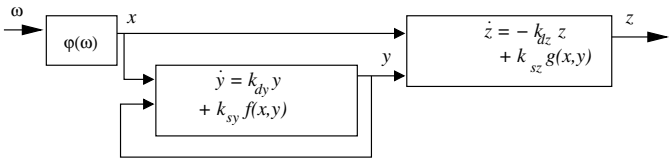


Fig. 3. Open loop λ switch experiment

Dodd et.al. have devised a means of breaking the loop, replacing the gene that encodes CI protein with one for LacZ protein, which does not interact with the Cro gene (Figure 2(b)). This technique enables measurement of LacZ as an output, given certain amounts of CI as input. CI is synthesised by means of another protein called the IPTG. In the experiment, the quantity of CI protein was measured indirectly. CI was made by introducing IPTG protein to a DNA ring (plasmid), separate to the plasmid containing the λ operators (essentially system of switches). A relationship between IPTG and CI was derived in a separate experiment comprising only the CI plasmid. All measurements were taken when the system was at steady–state.

Considering that inside *E. Coli* there are less than 100 copies of each molecular species [7], we need to take into account the intrinsic noise and employ stochastic modelling [18]. A continuous Markov model known as the Chemical Master Equation (CME) is a framework that has been used to model chemical reactions in low copy–numbers [8], such as used in ([10], [12]) the general representation of which is

$$\frac{\partial p}{\partial t} = \sum_j -w_j(\mathbf{x},t)p(\mathbf{x},t) + w_j(\mathbf{x}-\mathbf{z_j},t)p(\mathbf{x}-\mathbf{z_j},t),$$

where $p(\mathbf{x},t)$ is the probability of being in the state $\mathbf{x}$ of the system, w_j is the propensity function of reaction j, and $\mathbf{z}_j$ represents the stoichiometric change in chemical species due to reaction j.

To obtain the CME model of the λ-phage switch, we need to estimate the propensity functions $w_j(\mathbf{x},t)$, for which we have a mélange of information at hand: (relatively few) *in vivo* measurements, *in vitro* measurements ([16], [17], [20]), and qualitative knowledge of the λ-phage behaviour [15]. The *in vivo* measurements comprise only IPTG (from which we obtain CI) and LacZ values; no Cro measurements were made because they are experimentally difficult to perform [5]. The mixed nature of information at our disposal necessitates the use of a heuristic prior to statistical methods in order to estimate the propensity functions. The former is reported in this paper and the latter in [19].

II. MODELLING PROTEIN DYNAMICS

In this paper we postulate the propensitites of the synthesis rates of Cro and LacZ; their degradation rates are assumed to be simple first-order processes. We take approximations of the expectation of the CME model in order to obtain the equivalent kinetic-rate equations.

Let ω, x, y and z be the concentrations of IPTG, CI, Cro and LacZ respectively. The schematic in Figure 2(b) indicates

that x represses y, y represses z, and x both enhances and represses z. To be more precise, x and y self-repress above threshold x_{th} and y_{th}, and below x_{th}, x enhances synthesis of z. In the main experiment, the values of ω and z are measured at steady state. In a separate experiment, ω and x are measured to ascertain an empirical function of $x = l(\omega)$. From these, we can obtain a relationship between z and x. There are no measurements for y.

The dynamics of y and z can be expressed by

$$\frac{dy}{dt} = k_{sy} f(x,y) - k_{dy} y, \quad (1)$$

$$\frac{dz}{dt} = k_{sz} g(x,y) - k_{dz} z, \quad (2)$$

where $f, g > 0 \ \forall x, y$. The repressive and enhancing functions of the proteins can be summarised in the following properties:

x represses y	$\frac{\partial f}{\partial x} < 0 \ \forall x, y$
y self-represses above threshold	$\frac{\partial f}{\partial y} < 0 \ \forall x, y > y_{th}$
y represses z	$\frac{\partial g}{\partial y} < 0 \ \forall x, y$
x represses z above threshold	$\frac{\partial g}{\partial x} < 0 \ \forall y, x > x_{th}$
x enhances z below threshold	$\frac{\partial g}{\partial x} \geq 0 \ \forall y, x \leq x_{th}$

A. Formulation of protein synthesis functions

Existing models for synthesis of Cro and CI (or in this case LacZ) proteins are of the forms that are difficult to incorporate ([17], [20]) in the framework depicted in Figure 3; therefore we make educated guesses as to the functional forms for $f(x,y)$ and $g(x,y)$, based on biological qualitative knowledge described by the above conditions on the derivatives, models of other genetic switches and systems involving molecular cooperative binding in the literature ([6], [9], [13]).

The "dumbbell" shape of the CI protein leads to cooperative binding with itself when bound to the λ right–operator, resulting in self-enhancement when its level is relatively low [15], as expressed in the condition for $\frac{\partial g}{\partial x}$. We choose a function

$$\theta(x) = \frac{\alpha(\frac{1}{n} + \frac{1}{m})}{\frac{1}{n}(\frac{x}{x_{th}})^n + \frac{1}{m}(\frac{x}{x_{th}})^{-m}},$$

where $\alpha, n, m, x_{th} > 0$; the maximum α occurs at $x = x_{th}$. This shape is a variation of the Hill function, where the exponents m and n are related to the Hill coefficient, which is an indication of cooperative binding in biochemical reactions. The exponent m controls the steepness of the function when $x < x_{th}$, and n when $x > x_{th}$.

In the case where a protein represses the synthesis of another protein, the decreasing Hill function can be used,

$$\phi(x) = \frac{\alpha}{1 + (\frac{x}{x_0})^p},$$

where $\alpha, x_0, p > 0$, $\phi(x) = \frac{1}{2}$ at $x = x_0$, and p regulates the steepness of the function (the "hardness" of the switch). This formulation satisfies the conditions for $\frac{\partial f}{\partial x}$ and $\frac{\partial g}{\partial y}$. This function shape can also be used for self-repression by the Cro protein. Unlike the CI protein, Cro protein does not enhance its own synthesis before it represses itself [15].

We combine both frameworks, to postulate functions for the synthesis of proteins CI and Cro from Equations 1 and 2, as below,

$$f(x,y) = \frac{1}{[1 + (\frac{x}{x_0})^{\beta_1}][1 + (\frac{y}{y_{th}})^{\gamma_1}]} \quad (3)$$

$$g(x,y) = \frac{(\frac{1}{\beta_2} + \frac{1}{\beta_3})}{[\frac{1}{\beta_2}(\frac{x}{x_{th}})^{\beta_2} + \frac{1}{\beta_3}(\frac{x}{x_{th}})^{-\beta_3}][1 + (\frac{y}{y_0})^{\gamma_2}]} \quad (4)$$

where x_0, y_0 are indication of the repressive activity of x and y on another protein, e.g. at $x = x_0$, the synthesis rate of y is half its possible peak value. The thresholds x_{th} and y_{th} are the values at which the protein in question starts to repress itself. It can be shown in Figure 4 that the functions $f(x,y)$ and $g(x,y)$ satisfy the derivative conditions imposed by the enhancing and repressive relationships.

B. Estimating the parameters

We are now left with thirteen parameters $k_{sy}, k_{sz}, k_{dy}, k_{dz}, x_0, y_0, x_{th}, y_{th}, \beta_1, \beta_2, \beta_3, \gamma_1, \gamma_2$ to estimate. Approximate (*in vitro*) measurements of a few parameters can be found in the literature ([17], [16], [20]);

$\tilde{x}_0$	200-300 monomers	$\tilde{x}_{th}$	400-700 monomers
$\tilde{k}_{sz}$	4.11 monomers/min	$\tilde{k}_{sy}$	3.22 monomers/min
$\tilde{k}_{dz}$	$1.732 \times 10^{-2}\text{min}^{-1}$	$\tilde{k}_{dy}$	$4.042 \times 10^{-2}\text{min}^{-1}$

It should be noted that measurements x_0 and x_{th} are approximate, as there was no sensitive measure for the active λ repressor (x) [16]. The rate parameters were measured more accurately, but one should take into account that experiments were carried out *in vitro*, and hence cannot be simply adopted in the *in vivo* model. The rate parameters $\tilde{k}_{sy}$ and $\tilde{k}_{dz}$ are taken from the literature on CI, as in the experiment the LacZ gene sits in the place of the CI gene, and is attached to the operators and promoters affecting CI.

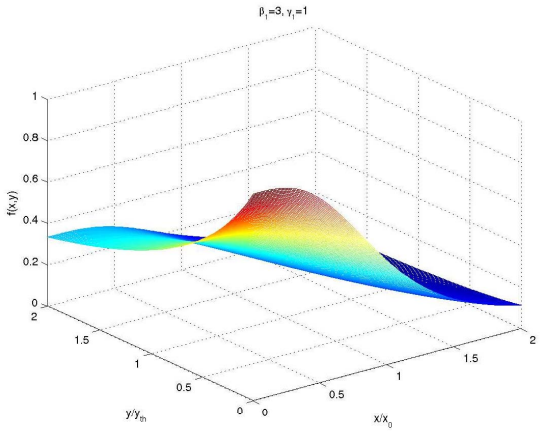
We now turn to the measurements of x and z, and our qualitative understanding of this system, to postulate the parameter values. The experiment was focused on the function of the CI protein, and it is unlikely that the produced Cro in the system has reached values $y \geq y_{th}$ that results in its own repression. It is safe to assume that in the operating region, $y << y_{th}$ which simplifies the expression for the synthesis of y to

$$f(x,y) \approx \frac{1}{1 + (\frac{x}{x_0})^{\beta_1}},$$

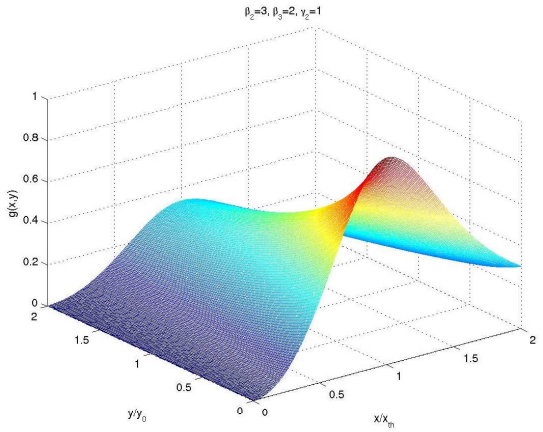
and reduces the number of parameters by two. At steady state, $y = \frac{k_{sy}}{k_{dy}} f(x,y) = \frac{K_y}{1+(\frac{x}{x_0})^{\beta_1}}$, where $K_y = \frac{k_{sy}}{k_{dy}}$. Substituting this into $g(x,y)$, we obtain

$$z = \frac{k_{sz}}{k_{dz}} g(x,y) = \frac{K_z(\frac{1}{\beta_2} + \frac{1}{\beta_3})}{[\frac{1}{\beta_2}(\frac{x}{x_{th}})^{\beta_2} + \frac{1}{\beta_3}(\frac{x}{x_{th}})^{-\beta_3}][1 + (\frac{\bar{K}_y}{1+(\frac{x}{x_0})^{\beta_1}})^{\gamma_2}]}, \quad (5)$$

where $K_z = \frac{k_{sz}}{k_{dz}}$ and $\bar{K}_y = \frac{K_y}{y_0}$. This manipulation yields a further reduction of three parameters.



(a) $f(x,y), x_0 = 1, y_{th} = 1, \beta_1 = 3, \gamma_1 = 1$



(b) $g(x,y), x_{th} = 1, y_0 = 1, \beta_2 = 3, \beta_3 = 2, \gamma_2 = 1$

Fig. 4. Shapes of the formulated synthesis functions

It is known that the CI protein dimer has a strong co-operative binding property, with itself and to the operators, and therefore $\beta_{1,3} > 1; \beta_2 + \beta_3 > 1$ and is likely to be closer to two, whereas Cro does not bind cooperatively, so $\gamma_{1,2} = 1$ or > 1 but is close to 1.

C. Fitting the curve for LacZ at steady state

We fit the function for z in Equation 5 to the data points (x_i, z_i), as shown in Figure 5, to obtain the following parameter values: $K_z = 120, \bar{K}_y = 0.1, x_{th} = 400, x_0 = 220, \beta_1 = 3, \beta_2 = 0.4, \beta_3 = 1.8, \gamma_2 = 1$. The parameter $\bar{K}_y$ affects the midriff kink around $x = 200$ in Figure 5, and γ_2 seems to have little effect. It is unclear whether the kink in the data plot has significance or whether it is merely due to rare measurements in that range. We can note that the curve fits well within the error bars of the data, in most of the range. The discrepancy sits in the low CI range, where the measurements indicate that at CI = 0, LacZ $\neq 0$. This affects the curve fitting in the low ranges (around CI < 50).

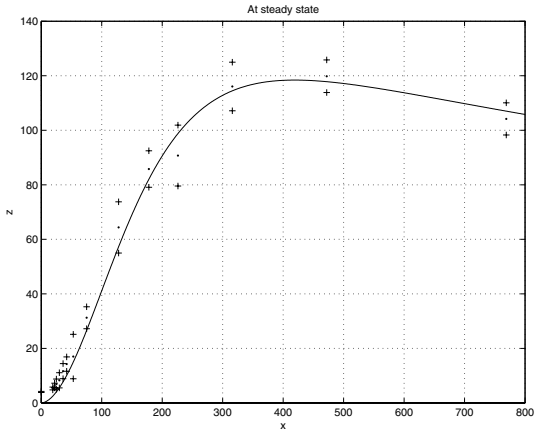


Fig. 5. LacZ vs CI, solid line = fitted curve, dot = average and + denotes error range

III. DYNAMIC SIMULATION

We re-closed the loop (in a sense "undid" the wet-lab experiment by Dodd, et. al.), and performed SIMULINK simulations to observe the dynamics of CI and Cro protein in the original system. In closing the loop, we need to introduce a non-unity gain such that a non-zero steady–state value for CI protein can be achieved. From Figure 5, we can see that closing the loop by equating LacZ = CI gives us only one stable steady state at CI = 0. This is important in validating this model, as the CI-dominant steady state is the path that the bacterium-phage symbiosis is likely to take, if the environment is favourable. This means that one LacZ unit corresponds to multiple CI monomers, which is consistent with biological observations [5] We find the critical gain (from Figure 5) to be approximately 2.14.

In [19], the Maximum Likelihood method is used to estimate the most likely rate constants, by comparing the marginal probability density function of z given x from the CME model to the histogram of the experimental data, such as illustrated in Figure 6. A value of $y_0 = 200$ (estimated to be close to x_0) is used. The following values of rate constants are found to have the greatest likelihood with respect to the

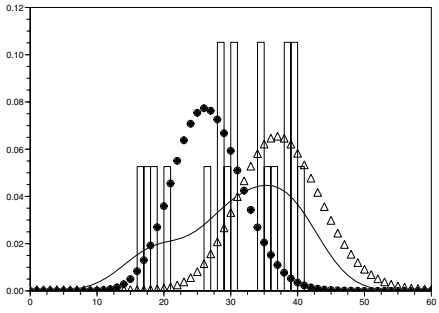


Fig. 6. Simulated distributions of LacZ given the *in vivo* parameters (△), parameters found by Maximum Likelihood (●) compared to the histogram of the observed data and its estimated pdf kernel (solid line), for $x = 75$

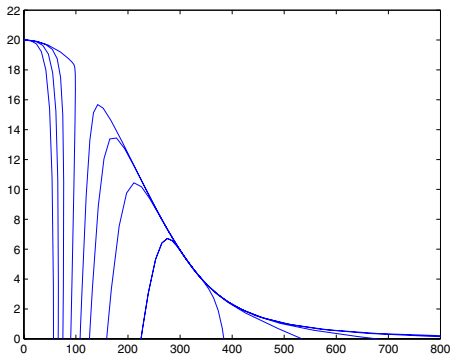


Fig. 7. Phase plot of Cro vs CI, with gain LacZ = 3CI, steady states (0,20),(310,5)

data: $k_{sy} = 3.213, k_{dy} = 0.161 k_{sz} = 9.11, k_{dz} = 0.0759$. We simulate using a gain of three (LacZ = 3 CI) to yield both steady states. We give initial values of $y(0) = 0$ and $x(0) \in \{19, 22, 25, 30, 36, 42, 53, 75, 128, 178, 226, 316, 472\}$, in accordance with the wet-lab experiment. We observe both steady states; when $x_{ss} < y_{ss}$ (lysis) and $x_{ss} > y_{ss}$ (lysogeny), as shown in Figure 7.

IV. DISCUSSION

Bistable switches such as the one found in the life–cycle of the bacteriophage-λ are widely–found motifs in gene regulatory networks. It is of interest to be able to construct a model of this switch, to be used as a module of a bigger network. Similar systems have been artificially constructed to study its approximate behaviour [6]. The λ-phage itself is well-known among the molecular biology community, having been studied since the 1950's. However, the models that have been constructed of its subsystems do not lend themselves to use in dynamical simulations, and the experiments have mostly been performed *in vitro*.

We describe above a heuristic for constructing a model of the λ bistable switch, from *in vivo* measurements. We use qualitative understanding of the biochemistry of the process[15] to postulate the formulation of the model, and to make approximations in estimating the parameters. We use also the *in vitro* measurements reported in the literature ([16], [17], [20], [21]) to aid the estimation process. Considering that there are only 153 measurements of z over 15 values of x, and 13 parameters to estimate, statistical methods of parameter estimation cannot be employed, leading us to turn to a heuristic. From this we manage to reduce the number of parameters by five, and fit a function to the data points. The remaining parameters are estimated using the Maximum Likelihood methods, against the histogram of the measured data [19].

Having postulated functional forms for the synthesis of CI and Cro proteins that satisfy the qualitative conditions of whether they enhance or repress protein (be this the other protein or itself) synthesis, we derived an expression of LacZ in terms of CI, based on the assumption that the protein Cro is unlikely to have reached levels at which it represses itself (as hinted in the literature), in Equation 5. We fitted this expression against the data points, yielding eight out of the 13 desired parameters. The approximation sheds two parameters y_{th} and γ_1, so to perform dynamical simulation we guess the values of synthesis and degradation rates, and y_0, from the values obtained in *in vitro* experiments.

We closed the loop with a gain, to simulate the original system (as in Figure 2(a)). The need for a non-unity gain revealed that one LacZ unit corresponds to multiple CI monomers (in fact, more than two), which was not indicated in the wet lab experimental measurements.

In its natural state, the λ-phage is a bistable system with the ability to switch from lysogeny to lysis but not vice versa. We had initially attempted to implement Angeli's sign test [1] to ascertain bistability of the model. However, the graphical representation of this λ-phage system includes an edge to which a definite sign cannot be assigned (Figure 2), due to the self–enhancive and –repressive behaviour of the CI gene. This renders us unable to use the sign test.

Our dynamical closed–loop simulation shows the existence of both stable states of the λ–phage, corresponding to lysogeny and lysis, but it is unable to demonstrate the switching behaviour from the former to the latter. This is to be expected from a deterministic model of this intra-cellular system. This is also shown in the simulation by Reinitz and Vaisnys [17], and remarked by Tian and Burrage [21].

The switching is caused by external factors (e.g., the presence of UV light) which affect the degradation rate of the CI protein. This needs to be incorporated into the stochastic model to simulate the switching. This has been demonstrated by Tian and Burrage [21] from an improvement of physico–chemical models ([17], [20]). Our work aims to produce an equivalent model more easily usable in the system–theoretic framework, hence enabling exploitation of system/control theory in the analysis of intra–cellular biological networks.

V. CONCLUSIONS AND FUTURE WORK

Modelling genetic networks is not a trivial matter, due firstly to the complexity of the system, and the lack of *in vivo* measurements for the rate parameters. An experiment is described here, where the closed loop is in a mutually–repressing toggle switch, yet the number of data points is still too few to allow statistical methods for parameter estimation. We couple the measured data and the qualitative knowledge of the system to estimate the parameters and to avoid problems of over-parameterisation.

We present a heuristic to estimate the dynamical equations for the two-state genetic switch from *in vivo* experimental results, guided by qualitative information on the protein properties and system behaviour and *in vitro* values from the literature. Taking into account the low copy number of chemical species, the full model needs to be stochastic; we are using a deterministic model as a stepping stone, to firstly estimate the parameters. Terms from this deterministic model are then used as propensity functions in the stochastic model [19].

This model displays the expected bistability but not switching. One needs to incorporate external noise in the stochastic model, which is currently a work in progress. It seems also that intrinsic noise in the system (due to low copy–numbers) dues plays an active role in the dynamical behaviour of the system, an issue which is under investigation.

VI. ACKNOWLEDGEMENTS

The authors wish to thank Ian Dodd and Keith Shearwin of the School of Molecular and Biomedical Science, the University of Adelaide, Australia, for the experimental data without which this paper could not have come into being, and many the fruitful discussions.

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