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Systems Biology and Control — A Tutorial

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INTRODUCTION

Cellular systems consists of large networks of interconnected molecular components involving considerable feedback, crosstalk and are highly nonlinear. To quote J. Michael Bishop, co-recipient of the 1989 Nobel Prize in Medicine and Physiology:

> To fully understand these pathways, we need a convenient and powerful model to complement the experimental research. Though there have been relatively few attempts to model signaling pathways using computers, it seems likely that this will very soon become a major area of study. [1]

Since writing this 12 years ago, progress in introducing mathematical and computational tools into biology has been phenomenal, leading to the emergence of a new field in Biology: Systems Biology.

A. Control engineering and systems biology

As might be expected, control engineering is playing a significant role in the new field of systems biology. Many of the "emergent" properties of signaling systems being discovered by biologists have been well-studied and analyzed by control engineers. The best example of this is robustness. In 1997 Naami Barkai and Stan Leibler published an influential paper in *Nature* [2] which argued that "The key properties of biochemical networks should be robust in order to ensure their proper functioning." So innovative was this concept to biologists that, in an accompanying article, Leland Hartwell (2001 Nobel Prize winner) states that Barkai and Leibler "have formulated a new concept, robustness, which has fundamental implications ..." [3]. It was through simple control-theoretic analysis, however, that this robustness was effected through a simple integral control feedback loop [4].

In this tutorial we introduce some of the concepts that frequently appear at the intersection of control theory and systems biology. Mustafa Khammash and Brian Munsky outline some of the key approaches for the modeling and analysis on cellular noise and the resulting fluctuations in the copy numbers of cellular constituents. Eduardo Sontag introduces some tools for analyzing deterministic biochemical reaction networks. Pablo Iglesias looks at models of spatially varying chemical reactions, and in particular, how gradients and patterns are formed in cells. Finally, Domitilla Del Vecchio considers important control-theoretic problems in synthetic biology — that is, the synthesis of basic circuits inside cells using genetic regulatory components.

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I. NOISE PROCESSES IN BIOLOGY — M. KHAMMASH AND B. MUNSKY

The cellular environment is abuzz with noise. A key source of this "intrinsic" noise is the randomness that characterizes the motion of cellular constituents at the molecular level. Cellular noise not only results in random fluctuations (over time) within individual cells, but it is also a source of phenotypic variability among clonal cellular populations. In some instances fluctuations are suppressed downstream through an intricate dynamical networks that acts as noise filters. Yet in other important instances, noise induced fluctuations are exploited to the cell's advantage. Researchers are just now beginning to understand that the richness of stochastic phenomena in biology depends directly upon the interactions of dynamics and noise and upon the mechanisms through which these interactions occur.

Tutorial Description: In this tutorial we review a number of approaches for the analysis of stochastic fluctuation, particularly those involved in gene expression.

Specific topics include:

- Origins and impact of cellular noise.
- Deterministic vs. stochastic models.
- Stochastic chemical kinetics. The Chemical Master Equation (CME).
- Monte Carlo methods. Gillespie's Stochastic Simulation Algorithm and its variants.
- Moment dynamics. Linear vs. nonlinear propensities.
- Linear noise and Langevin approximations.
- Direct methods for the solution of the CME.

A. Noise in biological networks

1) Noise origins and implications: Events in biological networks follow from reactions at the molecular level. The random nature of such reactions can be traced back to the random collisions among reactant molecules whose trajectories are driven by thermal motion. Such randomness leads to fluctuations in the molecular copy numbers of reactants both among similar cells and within a single cell over time. These fluctuations (commonly referred to as noise) can propagate downstream and impact events and processes in accordance to the dynamics of the network interconnection. Cellular noise has been measured experimentally and

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classified based on its source [5], [6]: intrinsic noise refers to noise originating within the boundaries of the process under consideration and is due to the inherent discrete nature of the chemical process of gene expression, whereas extrinsic noise has origins that are more global and affects all processes in the cell under consideration in a similar way (e.g. regulatory proteins copy numbers, RNAP numbers). Noise, both intrinsic and extrinsic, can play a critical role in biological processes. In [8], [9] it was proposed that

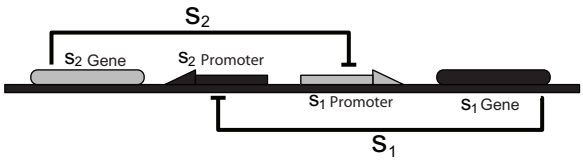

Fig. 1. Schematic representation of the Gardner-Cantor-Collins synthetic genetic toggle switch [7]. Protein s_1 suppresses the expression of the s_2 gene, while protein s_2 suppresses the expression of the s_1 gene.

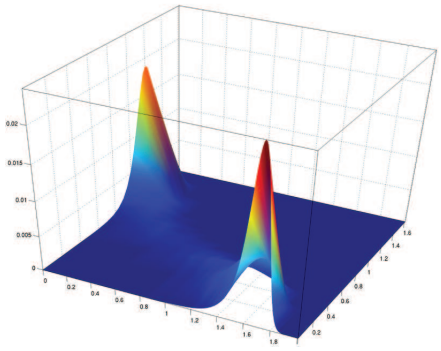

Fig. 2. The figure shows the bimodal nature of the distribution of proteins s_1 and s_2 in the genetic toggle switch shown in Figure 1. The distribution shown was computed using the Finite State Projection method described in sec. I-A.3.d

lysis-lysogeny fate decisions for phage λ are determined by a noise driven stochastic switch, implyng that the fate of a given cell is determinable only in a probabilistic sense. Another stochastic switch which governs the piliation of E. coli has been modeled in [10]. Aside from natural switches, bistable genetic switches have been constructed and tested [7], [11]. Depending on their parameters, such switches can be quite susceptible to noise. See also Figures 1 and 2. In [12], the first synthetic oscillator was reported. This novel circuit, called the repressilator, consists of three genes, each having a product that represses the next gene, thereby creating a feedback loop of three genes. The design of the repressilator was guided by a deterministic model as well as a discrete stochastic model that captures the effect of noise. The role of noise in the operation of the repressilator was recently studied in [13]. Another example where noise appears to play a important role involves the noise enhanced robustness of oscillations seen in relaxation oscillators (e.g. in the circadian rhythm). This effect, which is sometimes referred to as coherence resonance, has been studied in [14], [15]. Yet another curious effect of noise can be seen in the fluctuation enhanced sensitivity of intracellular regulation termed 'stochastic focusing' and reported in [16]. In gene expression, noise induced fluctuations in gene products have been studied in [17]–[26]. Many of these studies look at the propagation of noise in gene networks and the impact (and sometimes limitations) of various types of feedback in suppressing such fluctuations.

2) Deterministic vs. Stochastic Modeling: One approach to modeling reactions in biological networks uses the laws of mass-action, which results in a set of differential equations that describe the evolution of concentration of species adopted by the network over time. As an example, consider the reaction $A + B \xrightarrow{k} C$. A deterministic formulation of chemical kinetics would yield the following description $\frac{d[C]}{dt} = k[A] \cdot [B]$ where $[\cdot]$ denotes the concentration. Thus the concentration is considered a continuous variable. In contrast, a discrete stochastic formulation of the same reaction describes the *probability* that at a given time, t, the number of molecules of species A and B take certain integer values. In this way, populations of the species within the network of interest are treated as random variables. In this description, reactions take place randomly according to certain probabilities determined by several factors including reaction rates and species populations. For example, given certain integer populations of A and B, say N_A and N_B, at time t, the probability that one of the above reactions takes place within the interval $[t, t + dt)$ is proportional to $\frac{N_A \cdot N_B}{V} dt$, where V is the volume of the space containing the A and B molecules. Thus, in this mesoscopic stochastic formulation of chemical kinetics, molecular species are characterized by their probability density function which quantifies the amount of fluctuations around a certain mean value. In the limit of an infinite number of molecules (and infinite volume), fluctuations become negligible, and the mesoscopic description converges to the macroscopic description. In typical cellular environments where small volumes and molecule copy numbers are the norm, mesoscopic stochastic descriptions offer a more accurate representations of chemical reactions and their accompanying fluctuations. Such fluctuations need to be accounted for as they can generate distinct phenomena that simply cannot be captured by deterministic descriptions.

3) Approaches to Stochastic Noise Analysis:

a) The Chemical Master Equation and Monte Carlo methods: The Chemical Master Equation (CME) description accounts for the probabilistic nature of cellular processes. The CME describes the time evolution of the probability that the state of a reacting system consists of a certain number or concentration of molecules [27]. The CME can be derived based on the Markov property of chemical reactions. In this formulation, one considers a chemically reacting system involving N molecular species $S_1, ...S_N$ reacting through M reaction channels $R_1....R_M$. Let $X(t) = (X_1(t)....X_N(t))$ be the state vector, where

$X_i(t)$ is a random number that defines the number of molecules of species S_i in the system at time t. Assuming that the system is well stirred and in thermal equilibrium, each reaction channel R_k is a transition from some state $X = \mathbf{x}_i$ to some other state $\mathbf{x}_j = \mathbf{x}_i + \mathbf{s}_k$, where $\mathbf{s}_k$ is known as the *stoichiometric vector*. Associated with each reaction R_k is a *propensity function*, $w_k(\mathbf{x})$ which reflects the rate of the reaction k. Specifically, $w_k(\mathbf{x})dt$ is the probability that when the system is in state $X(t) = \mathbf{x}$, the k^{th} reaction will take place in a time interval $[t, t + dt)$.

The CME below describes the time-evolution of the probability that the system is in a given state $\mathbf{x}$ [27]:

$$\frac{\partial P(\mathbf{x}, t|\mathbf{x}_0, t_0)}{\partial t} = \sum_{k=1}^{M} [w_k(\mathbf{x} - \mathbf{s}_k)P(\mathbf{x} - \mathbf{s}_k, t|\mathbf{x}_0, t_0) - w_k(\mathbf{x})P(\mathbf{x}, t|\mathbf{x}_0, t_0)] \tag{1}$$

where $P(\mathbf{x}, t|\mathbf{x}_0, t_0)$ should be interpreted as the probability that $X(t) = \mathbf{x}$ at time t, given that $X(t_0) = \mathbf{x}_0$.

Because the CME is often infinite dimensional, the majority of analyses at the mesoscopic scale have been conducted using Monte Carlo algorithms. The most widely used of these algorithms is Gillespie's Stochastic Simulation Algorithm (SSA) [28]. Each step of the SSA begins at a time t and at a state $X(t) = \mathbf{x}$ and is comprised of three substeps: (*i*) generate the time until the next reaction; (*ii*) determine which reaction occurs at that time; and (*iii*) update the time and state to reflect the previous two choices. The SSA approach is exact in the sense that it results in a random variable with a probability distribution exactly equal to the solution of the corresponding CME. However, each run of the SSA provides only a single trajectory.

The biggest drawback of the SSA is that it is often prohibitively slow as it must step through one reaction at a time. One approximate accelerated simulation strategy is *tau-leaping* [29]. It advances the system by a *pre-selected* time τ which encompasses more than one reaction event. Roughly speaking, tau-leaping requires that τ be chosen small enough so that the following *Leap Condition* is satisfied: The expected state change induced by the leap must be sufficiently small that no propensity function changes 'appreciably' during that time. Tau-leaping has been shown to significantly speed up the simulation of *some* systems [29]–[32], but it is not as foolproof as the SSA. If one takes leaps that are too large, the tau leap assumptions may be violated and the results may be inaccurate or even nonsensical, e.g., some species populations might be driven negative. Moreover, if the system is "stiff", meaning that it has widely varying time scales with the fastest mode being stable, the Leap Condition will generally limit the size of τ to the time scale of the fastest mode, with the result that large leaps cannot be taken.

b) Langevin Approximation: Another approximation to the CME is the so-called Chemical Langevin Equation, which can be obtained through the tau-leaping multi-reaction update formula: Suppose that the leap-time τ can be taken small enough to satisfy the Leap Condition, but large enough that $w_k(\mathbf{x})\,\tau \gg 1$ for every reaction. While the Leap Condition holds, the number of reactions in the interval τ is a Poisson random variable with mean and variance equal to $w_k(\mathbf{x})\,\tau$. While this quantity is much larger than one, this random variable is well approximated by a normal random variable with the same mean and variance. This leads to the *Langevin leaping formula*

$$X(t + \tau) = \mathbf{x} + \sum_{k=1}^{M} \mathbf{s}_k w_k(\mathbf{x})\tau + \sum_{k=1}^{M} \mathbf{s}_k \sqrt{w_k(\mathbf{x})}\mathcal{N}_k(0, 1)\sqrt{\tau} \tag{2}$$

which expresses the state increment $X(t+\tau)-\mathbf{x}$ as the sum of two terms: a deterministic "drift" term proportional to τ, and a fluctuating "diffusion" term proportional to $\sqrt{\tau}$, where the $\mathcal{N}_k(0, 1)$ denote independent standard normal r.v. [33], [34]. From (2) one obtains the following (approximate) nonlinear stochastic differential equation, which is called the *chemical Langevin equation* (CLE) [33]–[35]

$$dX(t) \doteq \sum_{k=1}^{M} \mathbf{s}_k w_k(X(t))dt + \sum_{k=1}^{M} \mathbf{s}_k \sqrt{w_k(X(t))}dB_k(t),$$

where the $B_k(t)$ are independent standard Brownian motion processes.

c) Van Kampen's Linear Noise Approximation [36]: Another approximation that leads to a stochastic differential equation is the so called Van Kampen's approximation or Linear Noise Approximation (LNA) (see [25], [36]–[38]). It is essentially an approximation to the process $X(t)$ that takes advantage of the fact that in the large volume limit ($\Omega \to \infty$), the process $X^\Omega(t) := X(t)/\Omega$ converges to the solution $\phi(t)$ of the deterministic reaction rate equation: $\dot{\phi}(t) = F(\phi)$. Defining a scaled "error" process $V^\Omega(t) := \sqrt{\Omega}\big(X^\Omega(t) - \phi(t)\big)$ and using the Central limit theorem, it can be shown that $V^\Omega(t)$ converges in distribution to the solution $V(t)$ to the following linear stochastic differential equation:

$$dV(t) = J_F(\phi)V(t)dt + \sum_{k=1}^{M} \mathbf{s}_k \sqrt{w_k(\phi)}dB_k(t),$$

where J_F denotes the Jacobian of $F(\cdot)$ [39]. Hence, the LNA results in a state $X(t) \approx \Omega\phi(t) + \sqrt{\Omega}V(t)$, which can be viewed as the sum of a deterministic term given by the solution to the deterministic reaction rate equation, and a zero mean stochastic term given by the solution to a linear SDE. While the LNA is reasonable for systems with sufficiently large numbers of molecules (and volume), examples show that it can yield poor results when this assumption is violated, e.g. when the system of interest contains species with small molecular counts.

d) Finite State Projection: The authors have recently proposed a new analytical approach to solving the CME called the Finite State Projection (FSP) algorithm [40], [41]. The FSP approach relies on a projection that preserves an important subset of the state space (e.g. that supporting the bulk of the probability distribution) while projecting the remaining large or infinite states onto a single 'absorbing' state. Probabilities for the resulting finite state Markov chain can be computed exactly, and can be shown to give a lower bound for the corresponding probability for the original full system. The FSP algorithm provides a means of systematically choosing a projection of the CME, which satisfies any prespecified accuracy requirement. The basic idea of the FSP is as follows. In matrix form, the CME may be written as $\dot{\mathbf{P}}(t) = \mathbf{A}\mathbf{P}(t)$, where $\mathbf{P}(t)$ is the (infinite) vector of probabilities corresponding to each possible state in the configuration space. The generator matrix $\mathbf{A}$ embodies the propensity functions for transitions from one configuration to another and is defined by the reactions and the enumeration of the configuration space. A projection can now be made to achieve an arbitrarily accurate approximation as outlined next: Given an index set of the form $J = \{j_1, j_2, j_3, \ldots\}$ and a vector $\mathbf{v}$, let $\mathbf{v}_J$ denote the subvector of $\mathbf{v}$ chosen according to J, and for any matrix $\mathbf{A}$, let $\mathbf{A}_J$ denote the submatrix of $\mathbf{A}$ whose rows and columns have been chosen according to J. With this notation, we can restate the result from [40]: *Consider any distribution which evolves according to the linear ODE* $\dot{\mathbf{P}}(t) = \mathbf{A}\mathbf{P}(t)$. *Let* $\mathbf{A}_J$ *be a principle sub-matrix of* $\mathbf{A}$ *and* $\mathbf{P}_J$ *be a sub-vector of* $\mathbf{P}$*, both corresponding to the indexes in* J*. If for a given* $\varepsilon > 0$ *and* $t_f \geq 0$ *we have that* $\mathbf{1}^T \exp(A_J t_f)\mathbf{P}_J(0) \geq 1 - \varepsilon$*, then*

$$\| \exp(\mathbf{A}_J t_f)\mathbf{P}_J(0) - \mathbf{P}_J(t_f)\|_1 \leq \varepsilon,$$

which provides a bound on the error between the exact solution $\mathbf{P}_J$ to the (infinite) CME and the matrix exponential of the (finite) reduced system with generator $\mathbf{A}_J$. This result is the foundation for an algorithm to solve chemical reaction problems with guaranteed accuracy.

e) Moment Dynamics and Moment Closure: An important property of the Markov processes that describe chemical reactions is that when one constructs a vector μ with all the first and second-order statistical uncentered moments of the process' state X, this vector evolves according to a *linear* equation of the form

$$\dot{\mu} = A\mu + B\bar{\mu}. \tag{3}$$

Unfortunately, (3) is not a closed system because the vector $\bar{\mu}$ contains moments of order larger than two, whose evolution is not provided by (3) but are key to the solution to (3). A technique that can be used to overcome this difficulty consists of approximating the *open linear* system (3) by the following *closed nonlinear* system

$$\dot{\nu} = A\nu + B\varphi(\nu), \tag{4}$$

where ν is an approximation to the solution μ to (3) and $\varphi(\cdot)$ is a *moment closure function* that attempts to approximate the moments in $\bar{\mu}$ based on the values of the moments in μ. The construction of $\varphi(\cdot)$ often relies in postulating a given type for the distribution of X and then expressing the higher-order moments in $\bar{\mu}$ by a nonlinear function $\varphi(\mu)$ of the first and second-order moments in μ. Postulating a normal distribution is quite popular [42]–[44], but when the population standard deviations are not much smaller than the means, choosing $\varphi(\cdot)$ based on a normal distribution assumption often leads to bad approximations. Other authors construct moment closure functions $\varphi(\cdot)$ based on different assumed distributions for X, which include lognormal [45], Poisson, and binomial [46]. In [47] a new technique for moment closure that does not require *a priori* assumptions on the shape of the distribution for X has been proposed. Instead, the moment closure $\varphi(\cdot)$ is computed by trying to match all (or a large number of) the time derivatives of the exact solution to (3) with the corresponding time derivatives of the approximate solution to (4), for a given set of initial conditions. With this approach, it is indeed possible to match derivatives between (3) and (4) with small error [48]–[50]. Moreover, this can be done with moment closure functions $\varphi(\cdot)$ that do not depend on the (typically poorly known) parameters of the chemical reactions. This led to an automated methodology to construct the approximate closed systems (4). A set of MATLAB scripts that, constructs truncated moment dynamics given a set of chemical reactions may be found in [51].

II. Tools for the global analysis of deterministic biochemical networks — E. Sontag

Biomolecular networks, while exhibiting a rich variety of behaviors in signaling and regulation, would appear to be fairly well behaved as dynamical systems. Their (mathematical) models have solutions that tend to settle into well-defined steady states or periodic, but not "chaotic", behavior. This presents one major challenge to theoreticians: what is special about such networks, vis a vis general dynamical systems?

A second challenge arises in the mathematical analysis itself: while on the one hand good qualitative, graph-theoretic, knowledge is frequently available, on the other hand it is often hard to experimentally validate the form of the nonlinearities used in reaction terms, and even when such forms are known, to accurately estimate coefficients (parameters, such as kinetic constants). This "data-rich/data-poor" dichotomy seems to be pervasive in systems biology.

Our talk is concerned with both challenges. As an expository convenience, those approaches to the analysis of biochemical network dynamics to be described will be broadly classified as (a) *s/r-graph based* or (b) *i/o-component based*.

In the first category, we place those methods that exploit the fact that the structure of chemical reactions can

be specified by bipartite *species/reaction* graphs, leading typically to equations of the form $\dot{x} = SR(x)$, where S is a stoichiometry matrix and $R(x)$ is the vector of reactions (certain variants of this representation are also used). Included are results based on Feinberg-Horn-Jackson deficiency theory [52]–[64] as well as methods based on Petri net concepts [65]–[69]. These methods allow one to derive conclusions about chemical networks regarding stability, persistence, and number of steady states in ways that are extremely robust to parameter uncertainty.

In the second category, we place methods that are based upon the standard paradigm in control theory and signal processing, that of viewing larger systems as interconnections of input/output subsystems. Provided that these subsystems are individually well-behaved, more complex behaviors arise from the global interconnection structure. Included are results that assume that each component is passive (energy consuming in some abstract sense) and ask that interconnection graphs have appropriate diagonal stability structures [70]–[73], as well as results based on components being order-preserving (monotone) i/o systems [74]–[92]. Monotone components enjoy particularly nice dynamical properties as well as robust responses to perturbations. Their interconnections may be, in principle, studied through a blend of qualitative and (relatively sparse) quantitative i/o information, allowing one to draw conclusions about global dynamical behavior and the location of stable steady states.

III. Spatial heterogeneous models — P.A. Iglesias

A. Why spatial models arise

Implicit in any ODE model of biological systems is the assumption that chemical concentrations are spatially homogeneous. This assumption is valid for "well-stirred" systems in which the chemical species and related reactions are confined to small volumes. In many systems, however, spatial heterogeneities are present and play significant roles [93]–[102]. In particular, diffusion of biochemical molecules can play a significant role in biological regulation [103]. In these cases, ODE models such as

$$\dot{c} = f(c)$$

need to be replaced by reaction-diffusion equations of the form:

$$\frac{\partial c}{\partial t} = D\nabla^2 c + f(c)$$

Here, D is the diffusion coefficient of the interacting molecule. Note that stochastic models of spatial heterogeneous systems are possible, but can be extremely burdensome computationally; see [104]–[109] for a description and some examples.

B. Morphogen Gradients

Intracellular gradients play an important role in many cellular processes, including embryonic tissue development [99], [110], [111] as well as cell division [112]–[115] and motility [93], [116], [117].

One interesting way in which gradients can appear inside a cell is when an activator (eg. a kinase or guanine nucleotide exchange factor, GEF) is spatially restricted to be on a surface while its antagonist (eg. a phosphatase or guanine activating protein, GAP) is found in the adjoining cytoplasmic volume, where the activated protein is free to diffuse.

One example is the RanGTP system [112], [115], [118]–[121]. Ran is a small molecule that can be in an either active (RanGTP) or inactive (RanGDP) state. RCC1, the protein that converts RanGDP to RanGTP is found bound to the chromosomes, so that RanGTP production is restricted to the surface of chromosomes. In contrast, RanGAP, which hydrolyzes Ran (i.e., turns RanGTP to RanGDP) is freely diffusible in the cytosol. This spatially segregated regulation effects a RanGTP gradient where RanGTP is found preferentially near the chromosomes. This gradient is believed to aid in nuclear transport and in regulating the spindle checkpoint.

We can develop a quite simple model of how these gradients appear and how their shape is controlled [122], [123]. If we let $c(x,t)$ be the concentration of the activated species, then its concentration can be described by the reaction-diffusion equation

$$\frac{\partial c}{\partial t} = D\nabla^2 c - k_- c$$

where k_- is the rate at which the activated species is inactivated. To complete the description of the system requires that we specify the boundary conditions. Assume for simplicity that the system is one-dimensional, with finite length L. We assume zero flux at the boundary $x = L$. Moreover, we assume that the activating species is restricted to the surface $x = 0$, and that it produces c at a constant rate k_+. Thus, our boundary conditions are

$$\left.\frac{\partial c}{\partial t}\right|_{x=0} = k_+, \qquad \left.\frac{\partial c}{\partial t}\right|_{x=L} = 0.$$

This linear PDE can be solved analytically [122], [124]. If $c(x,0) = 0$, then

$$c(x,t) = k_+\lambda\frac{\cosh([x-L]/\lambda)}{\sinh(L/\lambda)} - \frac{k_+\lambda^2}{L}\sum_{n=-\infty}^{\infty}\tau_n\cos\left(\frac{n\pi x}{L}\right)e^{-t/\tau_n}$$

where

$$\lambda := \sqrt{D/k_-}, \quad \tau_n^{-1} := k_- + D\tfrac{n^2\pi^2}{L^2} \geq 0.$$

If we focus on the steady-state solution, we that the shape of the spatial pattern is governed by the size of the dispersion parameter, λ, relative to that of the domain of diffusion L. In particular, to get a steep gradient, requires that one of the following three requirements be met:

1) Small diffusion. In this case, the molecules do not diffuse far away during their lifetime and so remain near

the boundary at which they are activated. While it is difficult to vary the diffusion coefficient of a molecule in an aqueous medium, the *effective* diffusion coefficient can be varied by introducing barriers to diffusion. For example, the nuclear envelope ensures that RanGTP cannot easily diffuse into the cytosol, thus ensuring large ($\sim$ 500–1000 fold) differences in concentrations between the inside and outside of the cell [118], [119]. Even after nuclear envelope breakdown, the convoluted shape of the compacted chromatin restricts RanGTP diffusion thereby ensuring the presence of large gradients [112].

2) Large k_-. This ensures a small lifetime for the activated molecule and so limits its diffusion away from the boundary. This parameter allows considerable flexibility for regulation. In the RanGTP case, the k_- acting on RanGTP combines the effect of both RanGAP ($k \approx 5 \times 10^{-5}\,\mathrm{s}^{-1}$) and an auxiliary protein, Ran Binding Protein 1 (RanBP1) ($k \approx 3 \times [\mathrm{RanBP1}]\,\mathrm{s}^{-1}$, where $[\mathrm{RanBP1}]$ is the concentration of RanBP1 in μM Clearly, the presence of even nanoMolar concentrations of RanBP1 will increase the effective k_- for the reaction by several orders of magnitude, and greatly increase the steepness of the RanGTP gradient [112], [120].
3) Cell size. Large gradients require that L/λ be small. This can help explain how cell shape and size can regulate cell function without changes in the biochemical reaction network [100].

C. Turing patterns

Probably one of the best studied systems in biology in which spatial heterogeneities arise is the Turing pattern formation, also known as the Meinhardt/Gierer systems (hereinafter referred to as TMG systems) [125], [126]. The basic premise of TMG systems is that the interaction of chemical species with different dispersions can give rise to instabilities in a system that, without diffusion would otherwise be stable.

To see how these diffusion-driven instabilities arise, consider the following linear system of two species, u and v driven by the reaction-diffusion system

$$\frac{\partial}{\partial t}\begin{bmatrix} u \\ v \end{bmatrix} = A\begin{bmatrix} u \\ v \end{bmatrix} + \begin{bmatrix} D_u u_{xx} \\ D_v v_{xx} \end{bmatrix}. \tag{5}$$

Here, $A = \left[\begin{smallmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{smallmatrix}\right]$ is a stable matrix specifying the system reactions. The diffusion coefficients for u and v are D_u and D_v, respectively, and u_{xx} and v_{xx} are the partial derivatives along the one-dimensional direction.

It is straightforward to check [127] that the solution of the system is of the form

$$\begin{bmatrix} u \\ v \end{bmatrix} = \begin{bmatrix} \alpha_1 \\ \alpha_2 \end{bmatrix} \cos(qx) e^{\sigma t}.$$

Note that $q = 0$ gives rise to spatially homogeneous solutions which, because of the assumption on A, would be stable in the absence of diffusion.

For general q, one can check that (5) gives rise to the following quadratic equation specifying the relationship between σ and q:

$$\sigma^2 + \sigma\left(-a_{22} - a_{11} + (D_u + D_v)q^2\right) + \left((a_{11} - D_u q^2)(a_{22} - D_v q^2) - a_{21}a_{12}\right) = 0.$$

Note that, when $D_u = D_v = 0$ then this is purely the characteristic equation of A and so σ are the two eigenvalues of A which have negative real parts by assumption. However, in the presence of diffusion, instabilities may arise. In particular, if there exists q such that

$$h(q^2) := (a_{11} - D_u q^2)(a_{22} - D_v q^2) - a_{21}a_{12} < 0;$$

equivalently, if $h(q^2_{\min}) < 0$ where

$$q^2_{\min} = \frac{1}{2}\left(\frac{a_{22}}{D_v} + \frac{a_{11}}{D_u}\right) \tag{6}$$

then the homogeneous state-state may be unstable. Note that, once again, spatial dimensions will play a role, because the set of admissible q may be limited. If we assume that the region of interest is $x \in [0, L]$ then

$$q_n^2 = \frac{n\pi}{L}, \qquad n = 0, 1, \ldots$$

are only admissible. It may be the case that $h(q^2_{\min}) < 0$, but that $h(q_n^2) > 0$, $n = 0, \ldots$. However, as the size of the region is increased, the spacing between the q_n^2 decreases and so the likelihood that there exists an n such that $h(q_n^2) < 0$ is greater.

It is also worth noting how diffusive instabilities may arise [127], [128]. In particular, except for changes in the state order, one of the following two conditions must hold:

$$a_{11} > 0, \quad a_{21} > 0, \quad q_{12} > 0, \quad a_{22} < 0 \tag{7}$$

$$a_{11} > 0, \quad a_{21} < 0, \quad q_{12} > 0, \quad a_{22} < 0 \tag{8}$$

In the first case (7), chemical species x contributes to its own production ($a_{11} > 0$), as well as that of v: ($a_{21} > 0$). Similarly, v negatively regulates both u ($a_{12} < 0$) and v ($a_{22} < 0$). Thus, u and v are referred to as the *activator* and *inhibitor*, respectively. Moreover, (6) implies that the dispersion of u: $\lambda_u = \sqrt{D_u/a_{11}}$ must be smaller than that of v: $\lambda_v = \sqrt{D_v/|a_{22}|}$. For this reason u is the known as the *local* activator and v as the *global* inhibitor.

In the second possibility (8), production of u is activated by both its own presence ($a_{11} > 0$),and by the presence of v ($a_{12} > 0$). However, an increase in u reduces the concentration of v ($a_{21} < 0$). In this mechanism, v can represent a substrate, required for the formation of u, which, though global, is depleted.

D. Experimental evidence for Turing patterns

Though models based on Turing instabilities have been used to account for all kinds of spatially heterogeneous biological patterns, from tiger stripes [129, Chapter 3] and fish markings [130], to polarization in chemotaxing cells [131], most of these models tend to be fairly phenomenological,

and so considerable skepticism exists in the biological literature regarding its applicability to cellular signaling systems [132]. Recently, however, experiments guided by computational models based on Turing/Meinhardt/Gierer models has air follicle spacing in mice providing strong evidence for a genetic underpinning of a diffusion driven instability [133], [134].

IV. Synthetic biology — D. Del Vecchio

This tutorial presents an introduction to synthetic biology from a control systems perspective. It provides a description of the main objectives of synthetic biology, of the state of the art in such a field, of enabling technologies, and finally of the main techniques and challenges in the analysis and design of synthetic bio-molecular networks.

A. Introduction

Biologists have long employed phenomenological and qualitative models in order to help discover the components of living systems and to describe their behaviors. On the other hand, the analysis in living organisms of the dynamical properties of complex molecular reaction networks composed of interacting genes, mRNA, proteins, and metabolites requires a more quantitative and systems-level knowledge. Thus, in recent years the field of *systems biology* has emerged, whose focus is the quantitative analysis of cell behavior, with the goal of unraveling the basic dynamic processes, feedback control loops, and signal processing mechanisms underlying life, Complementary to systems biology is the engineering discipline of *synthetic biology*. The goal of synthetic biology is to extend or modify the behavior of organisms, and control them to perform new tasks [135]–[137]. Through the *de novo* construction of simple elements and circuits, the field aims to foster an engineering discipline for obtaining new cell behaviors in a predictable and reliable fashion. In the process, synthetic biology plays a role in improving the quantitative and qualitative understanding of basic natural phenomena. In fact, one approach to the testing of mathematical models of biological systems is to design and construct instances of the system in accordance to hypothesized models. Discrepancies between expected behavior and observed behavior highlight either research issues that need more studying, or knowledge gaps and inaccurate assumptions in models.

While tools from controls and dynamical systems theory, such as systems identification and robustness analysis, have been put to great use in systems biology for the analysis of naturally occurring biological systems, the use of this theory for the design of synthetic biological circuits is still emerging. The pioneering work of several biologists and physicists [7], [12], [19], [138], [139] shows the potential and the need for such tools when tackling the challenges of biological design. The experimental results of [138] and of [19] on a negatively auto-regulated gene agree with the mathematical predictions obtained by using straightforward feedback control analysis. However, more complicated systems such as the oscillators built in [12] and in [139] do not provide experimental results that match well the theoretical predictions. In particular, intrinsic and extrinsic noise sources [140] seem to disrupt the oscillating behavior of the repressilator [12]. In [139], the oscillations are only damped, which suggests that the parameters of the constructed system may not be inside the theoretically computed range of parameters that guarantee oscillations. From these results, the need emerges for *robust, model based design*.

Control and systems theory have much to offer to synthetic biology. But, conversely, one may look forward to new theoretical advances in control systems inspired by biological research. In particular, the standard control system paradigm of modeling a system as an input/output (dynamic) map may need to be revised when signals are carried by the physical displacement of molecules. Accordingly, the role of inputs and outputs may change when systems are interconnected. For discussion on this topic, the reader is referred to [141]–[144].

B. Enabling Technologies

The discovery of mathematical logic in gene regulation [145] and the early achievements in genetic engineering in the 1970s, such as recombinant DNA technology, set the stage for today's synthetic biology. Recent advances in molecular biology provide the ability to translocate and fuse promoters, operators, binding sites, and genes in almost any fashion on a size-wise-compatible plasmid through a procedure called *cloning*. Most importantly, a key enabler to synthesize DNA in amounts large enough to be used for transfection (or transformation) and for various measurement procedures has been the *Polymerase Chain Reaction* (PCR). This molecular biology technique allows a small amount of DNA to be amplified exponentially [146].

Another key enabling technology has been the development of *in vivo* measurement techniques that allow to measure the amount of protein produced by a target gene. For instance, green fluorescent protein (GFP) is a protein with the property that it fluoresces in green when exposed to UV light. It is produced by the jellyfish *Aequoria victoria*, and its gene has been isolated so that it can be used as a *reporter gene*. Other fluorescent proteins, such as yellow fluorescent protein (YFP) and red fluorescent protein (RFP) are genetic variations of the GFP. The reporter gene is inserted (cloned) into the chromosome, very close to the location of the target gene, so both are controlled by the same promoter. Since the target gene and the reporter gene are transcribed at the same rate, by measuring the intensity of the reporter gene light emitted one can estimate the concentration of the protein expressed by the target gene.

Just as fluorescent proteins can be used as a read out of a circuit, *inducers* function as external inputs that can be used to probe the system. Inducers function by disabling repressor proteins. Repressor proteins bind to the DNA strand and prevent RNA polymerase from being able to attach to the

DNA and synthesize mRNA. Two commonly used inducers are IPTG and aTc. Isopropyl-β-D-1-thiogalactopyranoside (IPTG) induces activity of beta-galactosidase, which is an enzyme that promotes lactose utilization, through binding and inhibiting the lac repressor. The anhydrotetracycline (atc) binds the wild-type repressor (TetR) and prevents it from binding the Tet operator.

For engineering a system with prescribed behavior, one has to be able to change the physical component features so as to change the values of the parameters of the model. This is now possible. For example, the binding affinity of a transcription factor to its site on the promoter can be weakened by base pairs substitutions. Protein decay rates can be increased by adding degradation tags at the end of the gene expressing the protein (*http://parts.mit.edu/registry/index.php/Help:Tag*). Promoters can be designed to accept multiple transcription factors (called combinatorial promoters) to implement regulation functions that accept several inputs [147]. This can be attained by combining the operator sites of several simple promoters [139].

C. Synthetic systems

Enabled by the recent technological developments briefly summarized in Section IV-B, a number of simple synthetic circuits with prescribed behaviors have been designed and built in *E. coli*. Naturally occurring transcriptional networks are very complex, however biologists have been discovering recurrent patterns of interconnection that appear frequently. These patterns are called *motifs* [148]. Thus, synthetic biologists have been focusing mainly on synthetically reproducing these network motifs to study their behavior in isolation with the hope to (1) understand their role and features and to (2) create a number of understood building blocks that can be interconnected to create more complex networks with predictable behavior. At the heart of this approach that constructs more complicated systems starting from simpler building blocks is the concept of *modularity*. This concept is briefly discussed in Section IV-D.

A self repressed gene. Negative autoregulation occurs when a transcription factor represses its own transcription [138]. This system has been fabricated and two major findings resulted from the measurements: negative autoregulation speeds the response time [19], and negative autoregulation promotes robustness to fluctuations in production rates [138]. By using standard linear control theory, one can immediately predict that an increased negative feedback increments the robustness of the equilibrium point with respect to fluctuations. The interesting part is that this result has been confirmed by experiments performed on a synthetic negative feedback loop cloned on a plasmid and then transformed in a bacterium. This fact is encouraging, as it means that the adopted modeling framework may be good enough to suggest design guidelines for circuitry to be implemented in the biological substrate.

The toggle switch. A genetic toggle switch is a bistable system in which reliable switches between the two steady-states are induced through an input signal. Any such genetic toggle switch typically needs particular behavioral characteristics in order to be considered a true "memory component". First, the toggle switch must exhibit bistability over a wide range of parameter values (transcriptional rates, translational rates, decay constants, etc.) that tend to fluctuate in a living cell. Second, the two steady-states must be highly tolerant of random fluctuations in molecular-species concentrations, so that noise-induced transitions between the two states are virtually non-existent [7].

In [7], an analysis is proposed based on the nullcline shape. Within appropriate parameter ranges, one unstable and two stable steady-states exist. At the stable steady-states, one of the repressors is dominant over the other, while the other repressor is shut down. A switch of the dominance-toggle is induced by externally repressing the dominant repressor so it cannot bind any longer to the target promoter for the other gene. The effect is to boost the expression of the formerly low-expressed repressor, which thus returns to its higher constitutive expression rate.

The relaxation oscillator. A relaxation oscillator can be obtained by virtue of the competition arising between a strong self-activating gene A that activates a repressor R and the repression of A by the repressor R. A genetic realization of this oscillator was proposed and fabricated by [139]. In order to obtain the parameter space that guarantees oscillations, a simple analysis based on a hybrid model was proposed by [139]. The analysis of a two dimensional model, which exploits the Poincaré-Bendixson Theorem, is proposed in [149]. The data obtained by the experiments in [139] show damped almost sinusoidal oscillations. The fact that the oscillations are damped means that the equilibrium point is stable (other behaviors are ruled out by the Poicaré-Bendixson Theorem). In [149], the analysis of a four dimensional model including the m-RNA dynamics is also proposed. The results suggest that the observed experimental oscillations are consistent with a parameter set close to a supercritical Hopf bifurcation [150] corresponding to a stable equilibrium point. This gives a clear suggestion of the required parameter change to obtain sustained oscillations.

The Repressilator. Elowitz and Leibler [12] constructed the first operational oscillatory genetic circuit in *E. coli* consisting of three repressors arranged in ring fashion, and coined it the "repressilator". The repressilator exhibits sinusoidal, limit cycle oscillations in periods of hours, which are slower than the cell-division life cycle. Therefore, the state of the oscillator is transmitted between generations from mother to daughter cells. Motivated by the analysis of cyclic feedback gene systems, Hastings [151] and Mallet-Paret and Smith [152] developed results which show that if the equilibrium point is unstable, the ω-limit set of any bounded trajectory is a periodic orbit. A detailed parametric analysis for the repressilator model, using Hasting's Theorem, can be found in [153].

Thus, one can search for parameter values to guarantee the instability of the equilibrium point. This procedure was followed by [12] in the design of the repressilator. The experimentally obtained oscillations are not dampened, suggesting that the parameter set was properly chosen and thus validating both the model and the analysis preformed. However, the oscillations appear to be very noisy compared to that of the relaxation oscillator in [139]. The causes of such a noisy behavior are currently under investigation [140].

D. Challenges for Control Systems Theory: The Modularity Assumption

In traditional systems theory, a system is usually modeled as an input/output device with internal dynamics. Such an input/output abstraction has been also implicitly employed to analyze the circuits described in the previous section. This input/output description has been very useful in several other fields of engineering, including mechanical and electrical engineering, for composing systems and for deriving properties of an interconnection by the properties of the composing systems. Such an abstraction, however, tacitly assumes that the input/output response and internal dynamics of a system does not change upon interconnection. Such a property of a system is commonly referred to as *modularity*. As it has been noticed by [141], viewing interconnections as input-to-output assignments and viewing signal transmission as unidirectional impose constraints that are not present in the physics of a system. Such constraints may be appropriate in special situations occurring in signal processing and electronics, mainly because such engineering systems have been on purpose designed to obtain unidirectional signal propagation. Natural physical and biological systems are not necessarily describable using such constraints. This is especially true in transcriptional networks, in which proteins are typically signal carriers that connect an upstream system to a downstream system. A protein that travels from an upstream system to a downstream one to, for example, act as a transcription factor of a target gene cannot participate to the network of interactions that characterize the upstream system. As a consequence, the fact that the protein travels to a downstream system to carry the signal will affect the behavior of the upstream system. From a systems and signals viewpoint, we can interpret this phenomenon by saying that a signal generated by the downstream system will travel upstream and affect the dynamics of the upstream system. We refer to this type of signal that travels form downstream to upstream as *retroactivity* [142]–[144]. The amount of such a retroactivity will change depending on the features of the interconnection and of the downstream system. For example, if the affinity of the promoter binding sites of the protein is low, one can expect that the fact that the protein acts as a signal carrier from upstream to downstream will not affect much the upstream system.

To formally model such a retroactivity phenomenon, it has been proposed [154] to define a system S to have internal state x, two types of inputs (I), and two types of outputs (O): an input "u" (I), an output "y" (O), a *retroactivity to the input* "r" (O), and a *retroactivity to the output* "s". In such a formalism, achieving low retroactivity effect becomes the control-theoretic problem of *disturbance attenuation* [154]. Other authors have proposed instead to re-define the inputs and outputs of components in a biomolecular system so as to attain zero retroactivity [143]. However, the feasibility of such an approach is still under discussion among researchers as it may rely on assumptions that not always are met in the biological substrate. Other approaches consider OPAMP [155] like schemes in order to attain low "output impedance" [156], while other researchers propose non-inverting amplifier-based schemes to decrease the retroactivity effect [154]. Such schemes, however, have still to be validated by *in vivo* implementation. Many open questions are left still to answer: To what extent can we perform modular design (as it is performed in electronics) by proper circuit design? What biological mechanisms can we exploit to achieve such a goal? Do natural biological systems employ mechanisms such as insulation to successfully propagate signals? Are there any input/output descriptions that result in lower retroactivity? These seem to be central questions to answer in order to develop a biological engineering discipline.

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