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STOCHASTIC APPROXIMATION FOR REGULATING CIRCADIAN CYCLES, A PRECISION MEDICINE VIEWPOINT

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ABSTRACT

Circadian cycles and other self-regulatory biological processes are the result of complex interactions between gene expression and molecular interactions that alternately produce activation or repression of chemical pathways. In this paper we study a Petri net simulation model that reproduces the cell behavior and use gradient estimation methods to find optimal input rates that result in a desired period for the cycle. The significance of our research is the potential early identification of pathologies that are caused by aberrant cycles, and the discovery of those rates that are of main importance for the control of the cycles. This viewpoint can enable specific cures for specific people, in accordance with personalized (or precision) medicine. We use SPSA as a stochastic approximation algorithm to drive the simulation to the optimal rates that result in a desired period and then propose a surrogate model for gradient estimation that evaluates the exact gradient for an "aggregate" system described by ordinary differential equations. The resulting estimators are then interpreted in terms of a conditional expectation given certain random times of events. Our hybrid model for gradient estimation addresses the problem of high dimensions and can potentially increase the efficiency of the optimization method by at least one order of magnitude.

1 INTRODUCTION

The recent announcement from the White House to allocate a budget of $215 million in 2016 for *precision medicine* acknowledges the need to increase research efforts to address the specific causes of disease, which may vary from person to person, rather than develop cures for the "average" person (MSNBC 2015).

Most of the research in this area falls within the scope of genomics and health informatics. In this work we focus on a specific problem, related to defects in self-regulatory mechanisms that keep the cells' biological clocks in order. Because of the daily periodic exposure to sunlight, cells must adjust to 24-hour patterns to function correctly. These are called *circadian cycles* (meaning "close to a day"). In mammals there is a master clock located in the in the hypothalamus that can function autonomously (without need of light exposure). The master clock can regulate other peripheral clocks via humoral signals and other chemicals. Peripheral circadian clocks are present in the liver, heart, lungs and kidneys, among other organs. It has been recently discovered (Sahar and Sassone-Corsi 2009) that aberrant circadian rhythm may cause tumorigenesis (when otherwise normal cells become cancer cells). Other mono-cellular mechanisms that rely on keeping good biological clocks are present in insulin regulation, sleeping patterns, etc.

The circadian mechanisms usually exhibit periodic patterns for the amount of certain molecules in the cell exhibiting peaks every 24 hours. Usually these patterns alternate for different molecules. As explained below, such patterns can be explained through the interaction of activation and repression mechanisms that affect these two types of molecules.

The mathematical model that we use in this paper describes a complete functioning circadian self-regulated mechanism for two molecules in a single cell. We use a stochastic process to model the number of molecules present in the cell. Various processes determine rates of production, destruction, binding and unbinding of various molecules in order to achieve the periodic behavior. Importantly, our model requires a set of 15 *rates* in order to reproduce the simplest circadian self-regulatory mechanism. The question that we address is the following. *When the rhythm is aberrant, what are the optimal rates that are required in order to restore the 24 hour cycle?*

We pose this problem as a stochastic approximation problem and solve it first using SPSA (Fu and Hill 1997). Then we propose a hybrid model to increase the efficiency in gradient estimation with potential increase in efficiency.

Establishing the optimal rates may provide indication for plausible treatment. Our model permits the use of stochastic simulation to first mimic the situation observed for the real biological system under study, and then to determine which rates should be increased or decreased to promote resetting of the clocks. Importantly, gradient estimation can provide insight as to which of the firing mechanisms is more important to restore the 24 hour cycle. How the rates can be controlled is outside the scope of our research for now and would require laboratory analysis of single cell systems. As an example, in (Seo, Park, Lim, Kim, Lee, Baldwin, and Park 2012) it was determined that both light and temperature may affect the circadian clock in plant cells. These are examples of *exogenous* stimuli that may help to modify the rates.

The paper is organized as follows. Section 2 presents a model for a self regulated clock. The chemical equations can be described in a fluid approximation as a set of differential equations or ODE's (Vilar, Kueh, Barkai, and Leibler 2002) that can provide information on an aggregate level. However when the number of molecules is small the fluid approximation is not justified. Section 3 explains the discrete event (Petri net) model that we use, following the ideas proposed by (Gillespie 1977). Section 4 states the optimization problem and simulation results using finite differences and SPSA. In Section 5 we present a stochastic fluid model approach for approximating the gradients in order to improve the accuracy.

2 BIOCHEMICAL MODEL FOR SELF-REGULATED CLOCK

We first review the basic mechanisms for gene expression and regulation. A *gene* is a functional segment of a DNA molecule that encodes the structure of a certain biomolecule: usually, the product is a protein, however it can be a functional RNA as well. The process of constructing a protein from its corresponding gene is called *gene expression*.

The first stage of this process is called *transcription*: at this stage the DNA segment that encodes the protein is copied to a new relatively short RNA molecule that is a called messenger RNA (or mRNA for short). After that, at the second stage called *translation*, the mRNA is translated into the protein. To sum up, for making a protein molecule, first, a mRNA is constructed, which becomes the blueprint for making the protein itself.

The rates at which the transcription process may increase or decrease when certain proteins (called *transcription factors*) bind to the gene. Two main types of gene regulation are **activation** and **repression**.

The gene regulatory network (GRN) consists of two genes D_A and D_R, the former is encoding a transcription factor A ("activator"), and the latter is encoding another protein that we call R ("repressor"). The genes are first transcribed into mRNA (we call them mA and mR) at corresponding rates α_A and α_R. The messenger RNAs mA and mR are then translated into corresponding proteins with the rates β_A and β_R.

The protein A is able to bind to the genes D_A and D_R, which is why we call it a transcription factor. Binding increases the rates of production to $\alpha'_A > \alpha_A$ and $\alpha'_R >> \alpha_R$ of their mRNAs, and the effect is particularly strong for the gene D_R. We use the notation D^b_A and D^b_R to represent the gene under binding.

The protein R, on the other hand, is not a transcription factor. It binds with the protein A making a complex C, which exists until the protein A in the complex degenerates. Figure 1 shows a diagram with the basic interactions that occur in the process.

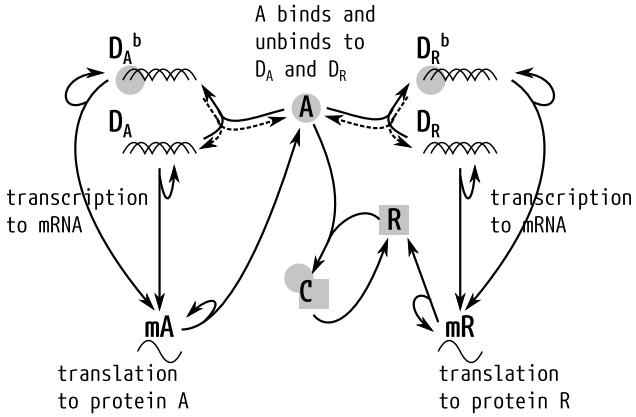


Figure 1: A diagram of the circadian clock model.

The following chemical equations state the various processes (Vilar, Kueh, Barkai, and Leibler 2002). We have also included actual values for the rates that we use later on in our simulation experiments.

Transcription (mRNA production):	D_A	$\xrightarrow{\alpha_A}$	$D_A + m_A$		
	D_A^b	$\xrightarrow{\alpha'_A}$	$D_A^b + m_A$		
	D_R	$\xrightarrow{\alpha_R}$	$D_R + m_R$	$(\alpha_R \approx 0)$	
	D_R^b	$\xrightarrow{\alpha'_R}$	$D_R^b + m_R$		
Translation (protein production):	m_A	$\xrightarrow{\beta_A}$	$m_A + A$		
	m_R	$\xrightarrow{\beta_R}$	$m_R + R$		
Binding and unbinding:	$D_A + A$	$\xrightarrow{\gamma_A}$	D_A^b		
	D_A^b	$\xrightarrow{\theta_A}$	$D_A + A$		
	$D_R + A$	$\xrightarrow{\gamma_R}$	D_R^b		
	D_R^b	$\xrightarrow{\theta_R}$	$D_R + A$		
Degradation:	m_A	$\xrightarrow{\delta_{m_A}}$	$\varnothing$		
	m_R	$\xrightarrow{\delta_{m_R}}$	$\varnothing$		
	A	$\xrightarrow{\delta_A}$	$\varnothing$		
	R	$\xrightarrow{\delta_R}$	$\varnothing$		
Binding of A and R:	$A + R$	$\xrightarrow{\gamma_C}$	C		
	C	$\xrightarrow{\delta_A}$	R		

γ_A	1.0
θ_A	50.0
γ_R	1.0
θ_R	100.0
α_A	50.0
α'_A	500.0
α_R	0.01
α'_R	50.0
β_A	50.0
δ_{m_A}	10.0
β_R	5.0
δ_{m_R}	0.5
γ_C	2.0
δ_A	1.0
δ_R	0.2

We use the common notation $[X]$ for the concentration of protein X in the cell. The ODE approximation by fluid dynamics describe the oscillations roughly as follows:

1. First, m_A are produced with the constant rate α_A, and then α'_A, but they decay at a rate δ_{mA}. The ODE approximation for this process is:

$$\frac{\partial[m_A]}{\partial t} = \alpha'_A - \delta_{mA}[m_A],$$

   which yields the steady state concentration of $[m_A] = \alpha'_A/\delta_{mA} \approx 50$. This happens almost immediately.
2. Next the slower growing production of mR (rate α_R at first and then α'_R) catches up, and eventually this mRNA gets to the steady state level $[m_R] = \alpha'_R/\delta_{mR} \approx 100$. Simultaneously, A gets to the steady state level, where

$$\frac{\partial[A]}{\partial t} = \beta_A[m_A] - \delta_A[A] = 0,$$

   reaching the magnitude $[A] = \beta_A[m_A]/\delta_A \approx 2500$.
3. On the other hand, mR produces the sufficient amount the protein R, which starts blocking the protein A, eventually depleting the activator protein A (the timing is a bit difficult to estimate here). This is the end of the cycle of A.
4. After that, there is still a considerable amount of m_R (we may assume it's still around the maximum steady state value 100), so there is active production of protein R and because there is almost no mA or A present in the system at this stage, $[R]$ grows. However, m_R degenerates exponentially fast.
5. Then, the remaining repressor proteins degenerate. Again, there is practically no mA or A present in the system, until R gets completely depleted, then the cycle repeats.

3 SIMULATION MODEL PETRI NET

The ODE model for chemical reactions can be a good approximation of an aggregate behavior when the number of molecules is high, thereby describing the dynamics of the concentration of material. However, in biochemical settings where the number of molecules may be very few, it is not always appropriate to use the concentration as a state description. An alternative model was introduced by (Gillespie 1977) assuming that particles are created or destructed individually, according to the given rates, as follows. When the state of the system (measured in occupancy or number of molecules of each of the various components) is X, there are a number of possible *events* that can happen either producing new particles, destroying particles or binding. Using a standard clock model for simulation, we assume that the time for next events are all independent exponential random variables with rates a_k (called *propensities*). Then the time for the next event has exponential distribution with rate $\sum_k a_k$, and the probability that the next event is event j is $a_j/\sum_k a_k$.

This model leads to a Petri net model for simulation shown in Figure 2 with corresponding algorithm:

- Initialize the number of molecules.
- Loop:
  - Compute the propensities a_k of each reaction.
  - Sample and fire the next reaction. $\mathbb{P}(j) = a_j/\sum_k a_k$.
  - Update time $t \leftarrow t + \Delta$, where $\Delta \sim \text{Exp}(\sum_k a_k)$.

Figure 3 shows the result of such simulation for a set of rates that yield an approximate 24 hour circadian cycle. There exist many tools for simulating biochemical reactions. We are using the program Beta Workbench (Dematté, Priami, and Romanel 2008) for running our model, then we process the output (an example of the output is shown in the Figure 3), computing an estimation of the expected period of the cycles of $[R]$, as well as an estimation of its variance.

To estimate the period P of the process we could in principle consider consecutive times at which $[R]$ reaches its midpoint from minimum to maximum level. However in the simulation there are many oscillations, so consecutive crossing points may be too close together and with high probability do not

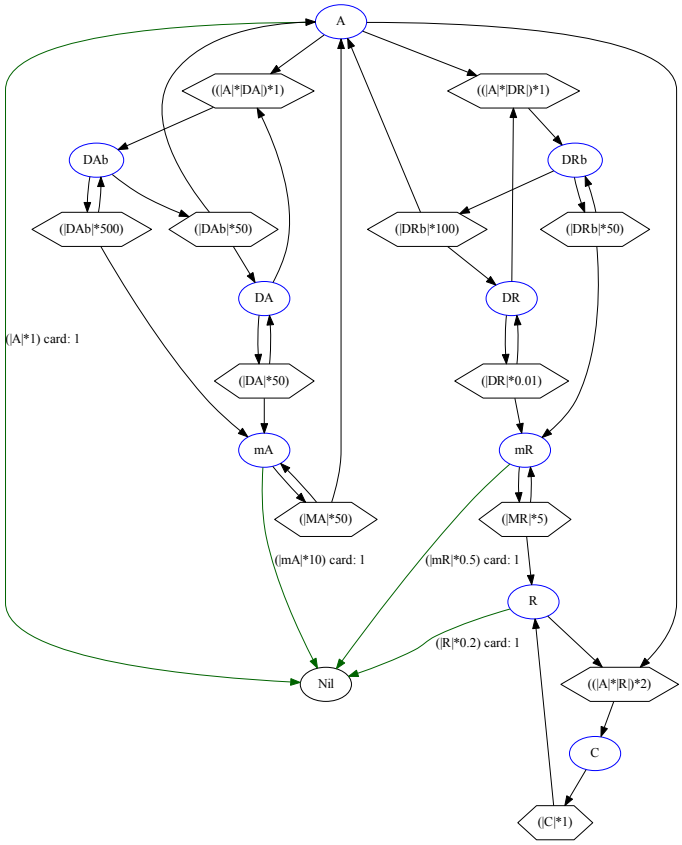


Figure 2: A Petri net of the circadian clock model generated by Beta Workbench

belong to different cycles, yielding a biased estimator of the period. Instead, we consider the first crossing time above a third of the maximum level and the first crossing time above two thirds of the maximum level. The we use a linear interpolation to solve for the expected crossing time for the midpoint. From our experiments, oscillations within a cycle are not large enough and the probability that $[R]$ crosses these two points within a single cycle is virtually zero.

Simulation of T hours then produces estimates $T(1), T(2), \ldots, T(N)$, where N is the (random) number of observed cycles within T hours. Then we use:

$$\hat{P} = \frac{1}{N-1} \sum_{n=2}^{N} (T(n) - T(n-1)).$$

Because the number N is random, this estimator is biased, but it is consistent as $N \to \infty$. Similarly, the variance is estimated with the sample variance. Because of the nature of the model, namely that residual times are memoryless and that cycles start with completely depleted concentrations of m_A, A, m_R and R, then we can assume that consecutive samples are (approximately) independent and identically distributed.

4 HYBRID MODEL FOR ESTIMATION

In order to improve the efficiency of the method we propose to use a hybrid model that approximates the discrete event Markov chain process with its expected or aggregate behavior dominated by ODE's only

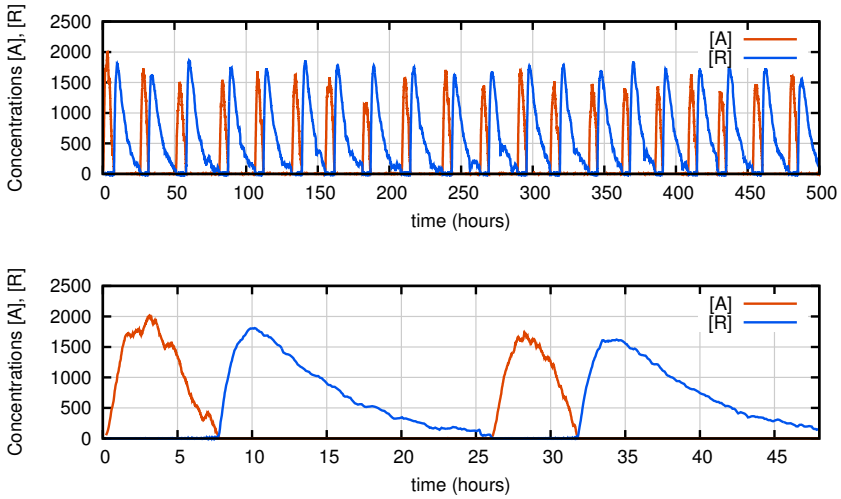


Figure 3: A simulation result with the rates that produce oscillations with the period approximately equal to 24 hours.

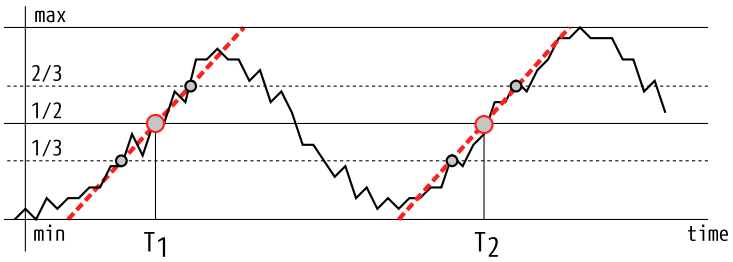


Figure 4: Illustration of the computation of expected period.

during certain subintervals within each cycle, avoiding the problem of "fractional" number of molecules that the full ODE model has.

Let $X(t) \in \mathbb{R}^5$ be the process that counts the number of molecules of each type. Specifically, let $X_1(t) = [A](t)$, $X_2(t) = [mA](t)$, $X_3(t) = [mR](t)$, $X_4(t) = [R](t)$, $X_5(t) = [C](t)$. Let $D(t) \in \{0,1\}^2$ denote the number of unbound DNA molecules D_A and D_R, so that $D(t) = (0,0)$ represents the case when both DNA's are bound. Notice that the rest of the molecules do not have an upper bound on their number and their dynamics follows a general multidimensional birth and death process. The model is a hybrid model because the rates are dependent on the *regime* dictated by the DNA component $D(t)$. Notice also that this component does not behave as a Markov Hidden Model, but it is dependent on the state $X(t)$.

Let k label de various possible events that either increase or decrease a component of X by one unit, and following Gillespie (2001) (**?**) let

$$\nu_{ik} = \begin{cases} +1 & \text{if event } k \text{ increases component } X_i \text{ by one} \\ -1 & \text{if event } k \text{ decreases component } X_i \text{ by one} \\ 0 & \text{otherwise.} \end{cases}$$

The *propensity* $a_k(X,D,\theta)$ is the corresponding rate at which event k occurs given state X,D and rates θ. For example, if $i = 2$ and k is the event of production of A then $a_k(X,D,\theta) = \beta_A X_1$, while for k' the event of degradation of A, we'll have $a_{k'}(X,D,\theta) = \delta_A X_2$. We use the notation $\mathfrak{F}_t$ for the natural filtration of the process, that is, $\mathfrak{F}_t = \sigma(X(s),D(s); s \le t)$.

Theorem 1 For any time T, conditioning on the event of no regime changes: $\{D(t+s) = D; s \leq T\}$ the process X satisfies:

$$\mathbb{E}(X_i(t+s) - X_i(t) \,|\, \mathfrak{F}_t) = \int_t^{t+s} \sum_k \nu_{i,k} \, \mathbb{E}(a_k(X(u), D, \theta) \,|\, \mathfrak{F}_t) \, du, \tag{1}$$

for any $s \leq T$.

Proof. Let $h > 0$ be an infinitesimal quantity. It follows from the exponential distribution and merging of Poisson processes that the probability of having two or more events within $[t, t+s)$ is $\mathscr{O}(h^2)$. The probability of no events is $1 + \mathscr{O}(h)$, and in this case $X(t+h) - X(t) = 0$. When there is only one event happening then $X_i(t+h) - X_i(t) = \nu_{ik}$ with a corresponding probability $h a_k(X(t), D, \theta) + \mathscr{O}(h^2)$, thus

$$\frac{\mathbb{E}(X_i(t+h) - X_i(t) \,|\, \mathfrak{F}_t)}{h} = \sum_k \nu_{i,k} \, a_k(X(t), D, \theta) + \mathscr{O}(h).$$

Given a constant regime D on $[t, t+T)$ the propensities are continuous functions of the state X. For given $h > 0$, let $t_0 = 0, t_n = t + nh$. Then using a telescopic sum,

$$\begin{aligned}
\mathbb{E}(X_i(t+s) - X_i(t) \,|\, \mathfrak{F}_t) &= \mathbb{E}\left(\sum_{n=1}^{\lfloor s/h \rfloor} (X_i(t_n) - X_i(t_{n-1}) \,\Big|\, \mathfrak{F}_t \right) + \mathscr{O}(h) \\
&= \mathbb{E}\left(\sum_{n=1}^{\lfloor s/h \rfloor} h \, \frac{\mathbb{E}(X_i(t_n) - X_i(t_{n-1}) \,|\, \mathfrak{F}_{t_{n-1}})}{h} \,\Big|\, \mathfrak{F}_t \right) + \mathscr{O}(h) \\
&= \mathbb{E}\left(\sum_{n=1}^{\lfloor s/h \rfloor} h \left(\sum_k \nu_{i,k} \, a_k(X(t_{n-1}), D, \theta) \right) \right) + \mathscr{O}(h) \\
&\xrightarrow{h \to 0} \mathbb{E}\left(\int_t^{t+s} \sum_k \nu_{i,k} \, a_k(X(u), D, \theta) \, du \,\Big|\, \mathfrak{F}_t \right).
\end{aligned}$$

□

It is interesting to note that if, and only if, the propensities are linear in the state X, then the so-called averaged process $< X(t) >$ satisfies the ODE

$$\frac{dx(t)}{dt} = \sum_k \nu_{i,k} \, a_k(x(t), D, \theta).$$

Gillespie mentions that this ODE cannot be accurate unless one looks at the limit when the number of molecules is large enough that one can approximate it by a continuous vector. We remark here that the ODE may be accurate for describing the *expected* behavior of the processes, provided that the propensities are linear, and that the regime is constant. For our particular model of the activator and represor proteins, there is a special regime when $D = (?, ?)$ when $a_k(X(t), D, \theta) = \gamma_C X_2(t) X_4(t)$ is the propensity for the increase of X_5 and decrease in both X_2 and X_4. Because of the correlations between components, in this case $\mathbb{E}(a_k(X(t), D, \theta) \neq a_k(\mathbb{E}(X(t), D, \theta)$.

The following plots show the stochastic processes for components X_2, X_4 and X_5 corresponding to the amount of A, R and C molecules. A full cycle can be divided into three distinct periods, each of which corresponds approximately to a different regime. The first part of the cycle describes the dynamics of A.

Assuming that $D = (,)$ during this period, it follows from Theorem 1 that the expected value of X_2 is the solution to the ODE:

$$\frac{dx_2(t)}{t} =, \tag{2}$$

because in this case the propensities are linear in the state X. It is possible to solve for the time $P_A = \inf(t \geq 0 : x_2(t) \leq 0)$ numerically.

The second part of the cycle describes the joint dynamics of R and C when there is no activator present. Alexey to calculate the steady state level of C for this part. The corresponding ODE is:

It is also possible to solve this ODE to find the time P_{RC} that it takes for the represor protein to reach the same level as the C compound.

The last part of the cycle is from the point where $x_4(t) = x_5(t)$ until there is production of A again. This part of the cycle has more complex dynamics, with non-linear rates and small amounts of molecules and regime changes that trigger the start of a new cycle. On this part we do not use the aggregate behavior, but the actual simulation of the process $\{X(t), D(t)\}$. The estimation of the period P can then be done using $\hat{P} = P_A + P_{RC} + \hat{P}_C$, where the last part is estimated with the Petri net model for the simulation.

5 GRADIENT ESTIMATION

5.1 Motivation

In this section we seek to determine the exact rates that result in a desired period, for instance 24 hour cycles. The input parameters or *control variables* are the 15 different rates that control the chemical reactions. We call θ the vector of rates. Notice that the propensities a_k are themselves functions of θ and of the current state of the process. The state describes the number of molecules of each kind as time evolves. In order to achieve a desired period we pose the optimization problem as a tracking problem, with

$$J(\theta) = \frac{1}{2}(P(\theta) - \pi)^2,$$

where π is the desired cycle time (such as 24 hours). The problem is then to minimize $J(\theta)$. Many tracking problems have a monotonic structure, where $P(\theta)$ is either decreasing or increasing in each of its arguments. In that situation it is common to use the Robbins Monro procedure directly with

$$\theta_{n+1} = \theta_n - M\varepsilon_n(\hat{P}(\theta_n) - \pi)$$

where $\{\varepsilon_n\}$ is a suitable step size sequence, and M is a constant diagonal matrix with ± 1 values on the diagonal, depending on the monotonicity of the function. However in our case the influence of rates in the increase or decrease of concentration of proteins is very complex and not necessarily monotonic. Thus it is necessary to use the information on the gradient of the period with respect to the rates.

Gradient estimation is also important in that the *sensitivity* to each of the rates θ provides information about the relative impact that each chemical process has. Rates with no statistically significant impact on the period should not be targeted for development of possible treatment.

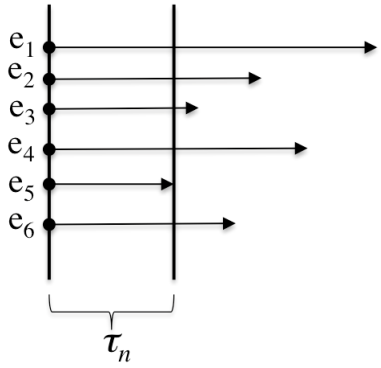
5.2 IPA Pathwise Estimation

In this section we present the theoretical results for application of IPA to the general model of the multidimensional birth and death process. Notice that the Gillespie model, which is a special case of the standard clock model for simulation (cite Vakili) generates one exponential residual time τ_n at iteration n and then generates the type of event following

$$\mathbb{P}(e_n = k) = \frac{a_k(X(t), D, \theta)}{\sum_m a_m(X(t), D, \theta)},$$

so e_n is a discrete random variable. With non zero probability, a small change in the rate vector θ may produce a different result for the chosen event. This in turn affects the future of the process in a discrete manner. This observation means that the model is not suitable for IPA methodology. find Glasserman's book!

Instead, we re-formulate the process within the canonical discrete event model where all residual times are exponential. The model has the same distribution as the Petri net model. For this section, we use $Y(t) = (X(t), D(t))$ for the state vector, and we call $E(y)$ the set of possible events when at state y.



At the time of an event the new state Y is obtained and all residual clocks C_k are defined by

$$C_k(Y,\theta) = -\frac{1}{a_k(Y,\theta)} \ln(U_{n,k}),$$

where $\{U_{n,k}\}$ are iid uniform random variables. The figure shows an example with six possible events. The time to the next event as well as the next event type are determined by the minimum:

$$\tau_n = \min(C_k(Y,\theta), k \in E(Y)),$$
$$e_n = \arg\min(C_k(Y,\theta), k \in E(Y)).$$

The next state $Y(t+\tau_n)$ is determined by the increase or decrease of component values, according to the quantities ν_{i,e_n}. By construction, because the minimum of independent exponential variables is also exponential with a rate that is the sum of the individual rates, it follows that this model (albeit not efficient for simulation) is equivalent to our simulation model, that is, the corresponding process has the same distribution as the original one. The time of the n-th event is $T(n) = \sum_{m=1}^{n} \tau_m$. Finally we denote by $\{Y_n\}$ the embedded process, that is, the values of the process $Y(T_n)$ sampled at the times of events.

Let the end of the cycle be defined by the random stopping time:

$$N(\theta) = \min(n\colon X_2(T(n)) > \ell),$$

where $\ell \geq 0$ is the minimal level in the sub period where the activator protein A dominates the process. This is where the cycles end, and a new one starts. Then the period length is

$$P(\theta) = T(N(\theta)) = \sum_{n=1}^{N(\theta)} \tau_n.$$

Lemma 1 Let $(\Omega, \mathbb{P})$ denote the probability space where the process $\{Y_n\}$ is defined as a function of the uniform random sequence $\{U_{n,k}\}$. The random variable $T(\omega,\theta)$ is locally Lipschitz continuous at θ w.p.1. and has a Lipschitz constant with bounded expectation.

Proof. Without loss of generality, we show the result for an arbitrarily chosen component j of the vector θ. Let $\Delta\theta \in \mathbb{R}$ be sufficiently small and call $\theta' = \theta + \Delta\theta$ the rate vector that has same components as θ except for the j-th component where the infinitesimal quantity Δ has been added. We need to show that there is a random variable L such that

$$|P(\theta') - P(\theta)| \leq |\Delta\theta| L,$$

and $\mathbb{E}(L) < \infty$. Given $\Delta\theta$ the measure space is partitioned: $\Omega = \Omega^c \cup \Omega^s$, where Ω^c contains all trajectories where there is no event swapping when using θ' instead of θ. □

5.3 Hybrid Gradient Estimation

6 STOCHASTIC OPTIMIZATION VIA SIMULATION

Include perhaps a discussion comparing the method with SPSA.

7 CONCLUDING REMARKS

We are currently implementing the hybrid model for the gradient estimation. Preliminary calculations show dramatic increase in efficiency compared to the SPSA procedure. We expect to have all the results ready for publication within the month, to be included in the final version of the paper.

The hybrid model is similar to the stochastic fluid models (SFM) (Wardi, Melamed, Cassandras, and Panayiòtou 2002, Melamed, Pan, and Wardi 2007). These models were proposed for queueing networks and approximate the dynamics of single events by "fluid" dynamics, but moderated by slower time scale events that change the rates of the fluid vessels. To express the circadian process as a full blown SFM would be very complex, because contrary to existing examples, in our model the propensities that trigger regime changes for the DNA binding depend in a complex manner on the state of the system. This is the subject of future research.

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OLD MATERIAL:

The optimization approach that we followed is an application of SPSA (He, Fu, and Marcus 2003), which ensures almost sure convergence to θ^* when minimizing $J(\theta)$, provided that this is convex.

At each iteration n, we sample N random perturbations $(\Delta_n)_1 \ldots (\Delta_n)_N$ uniformly from the set of two possible values $\{+1, -1\}$, and the magnitude of these perturbations is given by the formula $c_n = const/(n+1)^{0.101}$. This value for the decreasing parameter is shown to be optimal for better convergence rate in (Bhatnagar, Prasad, and Prashanth 2013). At each iteration n of the SPSA algorithm we simulate the biological system for $T = 500$ hours, at rate $\theta_n + c_n\Delta_n$ and in parallel we also simulate $T = 500$ hours at rate $\theta_n - c_n\Delta_n$. The gradient is then approximated by the symmetric finite difference:

$$g_n = \frac{\hat{J}(\theta_n + c_n\Delta_n) - \hat{J}(\theta_n - c_n\Delta_n)}{2c_n}$$

then we update each of the parameters:

$$(\theta_{n+1})_i = (\theta_n)_i - \varepsilon_n \frac{(g_n)_i}{(\Delta_n)_i}$$

where $\varepsilon_n = const/(n+2)^{0.602}$. The value for the decreasing step size is shown to be optimal in (Bhatnagar, Prasad, and Prashanth 2013).

Figure 5 shows the result of the SPSA for a target cycle of $\pi = 48$ hours. Observe that the most important rate turns out to be δ_R, the degradation rate of the protein R, which is consistent with the model: The decay of R has a longer tail now, and so the period increases.

In order to get insight into the sensitivity of the input rates, simulations were performed at constant θ to estimate the gradient via finite differences.

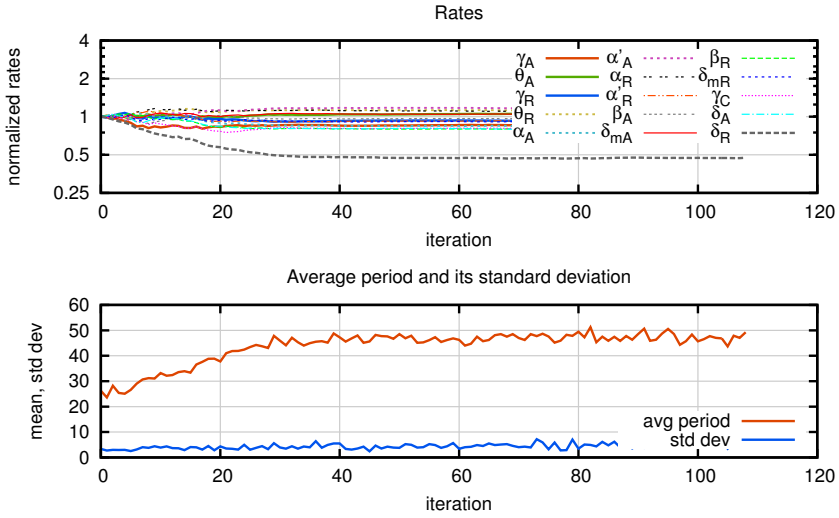

Figure 5: SPSA iteration procedure is convenrging to the oscillations

Derivatives of expected period $\mathbb{E}(P(\theta)$

	γ_A	θ_A	γ_R	θ_R	α_A	α'_A	α'_R	α'_R	β_A	δ_{mA}	β_R	δ_{mR}	γ_C	δ_A	δ_R
Mean deriv	-2.4	-3.9	-0.047	-0.44	1.2	8.2*	-3.6	-3.5	1.7	-1.8	-2.3	-7.1*	6.0*	-5.0*	-20.6**
StdErr of mean	3.1	3.2	3.0	3.2	3.0	3.4	2.9	3.2	3.3	2.9	3.1	3.0	3.1	2.9	3.0

Derivatives of variance of period $\mathbb{E}(P(\theta)$

	γ_A	θ_A	γ_R	θ_R	α_A	α'_A	α'_R	α'_R	β_A	δ_{mA}	β_R	δ_{mR}	γ_C	δ_A	δ_R
Mean deriv	9.7	6.7	8.8	31*	-2.7	11	11	-0.97	-10	-14	-15	-7.4	-11	-8.1	-18
StdErr of mean	12	15	15	16	13	14	14	17	13	14	15	17	14	14	14

The numbers i the tables agree with our assessment that very few rates have a significant effect in the period. Those are signaled by an asterisk, and represent the input rates that have a non-zero derivative with statistical significance. For completeness we have also included the computation of the derivatives of the variance of the period. The variance of the period is an important quantity because when large, it describes possible malfunction due to arrhythmic cycles. Estimation of the gradients and variances may help to identify such scenarios as well as possible intervention remedies.

Results from the simulations show that the general methodology of stochastic approximation works in order to determine optimal rates to achieve a desired target cycle time. However more realistic system will involve many other molecules and interactions, as well as possible exogenous agents and the dimension of the control vectors may be unwieldy.

To explain our approach, Figure 6 shows a trajectory of a system with a 48-hour cycle. In the bottom panel one single typical cycle is plotted. The red curve represents the amount of protein A and the blue, the amount of R. The cycle time $P(\theta) = P_A(\theta) + P_R(\theta)$ is the sum of two quantities: one representing the amount of time when there is no R present (but many just A) and the other is the amount of time that $[R]$ is non zero.

We posit that the cycle time's sensitivity to the rates *depends mainly* on the part $P_R(\theta)$. That is, we use the approximation

$$\nabla_\theta P(\theta) \approx \nabla_\theta P_R(\theta).$$

To estimate $P_R(\theta)$, this interval can be divided into two sections. The first can be described by the equation of the exponential decay of m_R

$$[m_R](t) = [m_R](0) \cdot \exp(-\delta_{mR} t) \approx \frac{\alpha'_R}{\delta_{mR}} \exp(-\delta_{mR} t),$$

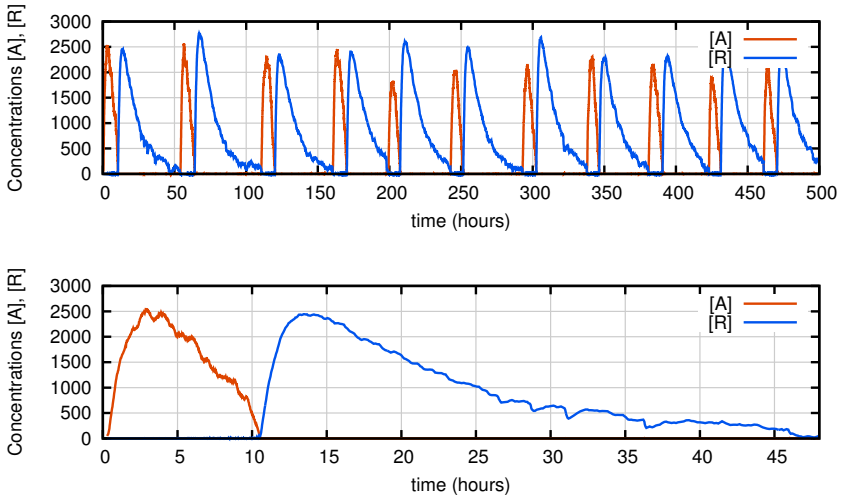

Figure 6: A simulation result with the rates that produce oscillations with the period approximately equal to 48 hours.

here we can assume that the initial concentration of $[m_R](0) = \alpha'_R/\delta_{mR} \approx 50$, it is still at the steady state concentration level. Simultaneously, R is also get produced:

$$\frac{d[R](t)}{dt} = [m_R](t)\beta_R - \delta_R[R](t),$$

which is a linear differential equaltion, and it can be solved for $[R](t)$, then the first stage ends approximately at the moment when $\frac{d[R](t)}{dt} = 0$, i.e. when $[m_R](t)\beta_R - \delta_R[R](t) = 0$. So the duration of this stage can be calculated in terms of this conditional expectation. After that, the second stage of a simple exponential decay of R, which lasts until $R[t] \approx 1$. Thus we can estimate the duration of both stages, and so calculate $\nabla_\theta P_R(\theta)$ as a function of the rates.