Modeling Control of Supercoiling Dynamics and Transcription Using DNA-Binding Proteins

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***Abstract*—Nearly all natural and synthetic gene networks rely on the fundamental process of transcription to enact biological feedback, genetic programs, and living circuitry. In this letter, we investigate the efficacy of controlling transcription using a new biophysical mechanism, control of localized supercoiling near a gene of interest. We postulate a basic reaction network model for describing the general phenomenon of transcription and introduce a separate set of equations to describe the dynamics of supercoiling. We show that supercoiling and transcription introduce a shared reaction flux term in the model dynamics and illustrate how the modulation of supercoiling can be used to control transcription rates. We show the supercoiling-transcription model can be written as a nonlinear state-space model, with a radial basis function nonlinearity to capture the empirical relationship between supercoiling and transcription rates. We show the system admits a single, globally exponentially stable equilibrium point. Notably, we show that mRNA steady-state levels can be controlled directly by increasing a length-scale parameter for genetic spacing. Finally, we build a mathematical model to explore the use of a DNA binding protein, to define programmable boundary conditions on supercoiling propagation, which we show can be used to control transcriptional bursting or pulsatile transcriptional response. We show there exists a stabilizing control law for mRNA tracking, using the method of control Lyapunov functions and illustrate these results with numerical simulations.**

***Index Terms*—Biomolecular systems, biological systems, nonlinear control.**

I. Introduction

CONTROLLING the magnitude and rate of gene transcription from DNA into mRNA is a fundamental problem in systems and synthetic biology [1], [2], [3], [4], [5], [6], [7]. At the single cell level, controlling gene transcription is a problem of controlling discrete molecular states, which manifests empirically as a stochastic control problem [7], [8], [9], [10], [11]. At the population level, controlling gene transcription is a problem of controlling the distribution of mRNA counts across a population of cells, i.e., a distributional control problem [9]. More frequently, the problem is formulated in terms of the control of the first moment (the mean) of the population's gene transcription or the centered second moment (the variance) [9].

There are many methods for controlling gene transcription. For example, transcription factors can be programmed to activate or repress the promoter of a gene, which has the effect of turning gene transcription on or off [12], [13], [14], [15], [16]. Further, CRISPRi control uses sequence-programmable targeting RNA molecules to direct the binding of CRISPR proteins to promoter, ribosome binding sites, or within a transcriptional reading frame, to sterically occlude the procession of polymerase enzymes [17], [18], [19], [20]. Both transcription factor and CRISPR-based control are limited by the slow rate at which proteins are translated and folded. More recently, researchers [4] have shown that optogenetic proteins with light-responsive domains can transduce *ex vivo* sourced light into intracellular control signals, thereby enabling design and execution of transcriptional control through an *in silico* controller. Genomic DNA is typically double-stranded and modeled as a double-helical structure, averaging about 10.5 basepairs per rotation or turns in the genetic sequence [15]. The number of rotations r in a double stranded DNA fragment of length n is referred to as the linking number of DNA. The linking number normalized by the length of the DNA fragment (r/n) is called the supercoiling density (σ) of the DNA [21]. In [21], it was shown that transcribed DNA generates two, distinct domains of supercoiling. Upstream of the promoter of a gene is a relaxed domain of negatively supercoiled DNA (DNA rotating in the left-handed direction). Downstream of the gene is a hypercoiled region of positively coiled DNA, rotating in the right-handed direction. Extensive accumulation of twist of either type can result in *writhing of the DNA*, where the backbone of the hypercoiled DNA accommodates further torsional stress by rotating in 3D intracellular space. Higher order twisting in 3D space is referred as writhing and excessive writhing can form higher-order knots in DNA known as plectonemes.

Manuscript received 8 March 2024; revised 3 May 2024; accepted 18 May 2024. Date of publication 27 May 2024; date of current version 24 September 2024. This work was supported by the Research Funds of Mokpo National University in 2023. This work was supported in part by the NSF CAREER Award under Grant 2240176; in part by the Army Young Investigator Program Award under Grant W911NF2010165; in part by the Institute of Collaborative Biotechnologies/Army Research Office under Grant W911NF19D0001, Grant W911NF22F0005, Grant W911NF190026, and Grant W911NF2320006; and in part by the Subcontract Awarded by the Pacific Northwest National Laboratory for the Secure Biosystems Design Science Focus Area "Persistence Control of Engineered Functions in Complex Soil Microbiomes" sponsored by the U.S. Department of Energy Office of Biological and Environmental Research. Recommended by Senior Editor S. Olaru. *(Corresponding author: Enoch Yeung.)*

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Digital Object Identifier 10.1109/LCSYS.2024.3406268

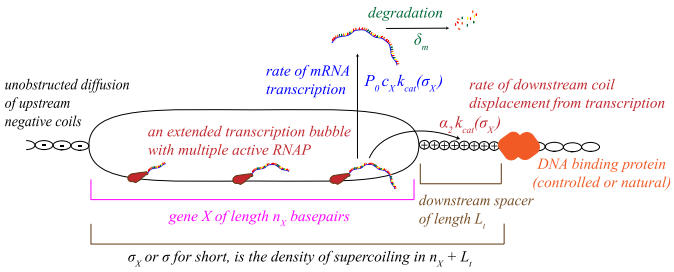

Fig. 1. A schematic illustrating the scenario of interest: we explore the effect of trapping supercoiling with DNA binding proteins immediately downstream of a single gene of interest (see reactions (1-5)).

In the presence of extensive torsional stress, the degree of supercoiling near a gene can directly control transcription. In [22], [23], researchers showed that high levels of positive supercoiling can quench gene transcription. Each time a gene transcribes with a higher degree of activity, it accumulates enough positive supercoiling to self-repress to an off-state [22]. The overall effect is that RNA transcription will occur in pulsatile bursts, or bursty transcription [24]. Thus, DNA supercoiling is a fundamental component of transcriptional bursting [22]. Control strategies utilizing supercoiling to alter the frequency or magnitude of transcriptional bursting could be used to alter cell fate [25], genomic programs [15], and pathogenesis in various species of bacteria [26].

Here we develop novel, mathematical models for a biophysical approach to transcriptional control. We investigate the feasibility of controlling a single gene's transcription by controlling the supercoiling around the gene using interference from a DNA binding protein [17]. Specifically, we model utilization of a DNA-binding protein with sequence-directed specificity. There are DNA binding proteins with defined recognition sites, e.g., CRISPR or Par protein systems that utilize targeted binding to a gene sequence. Utilizing a mathematical approach, we explore in this letter whether a DNA binding protein, could be used to control the local supercoiling state surrounding a gene [14], to *both upregulate or downregulate* gene transcription.

II. Transcriptional Dynamics With Supercoiling

To begin we consider transcription of the mRNA m_X of a single gene X of length n_X basepairs, with transcription rate dependent on the local supercoiling density σ_X. We assume the mRNA is subject to degradation and the supercoiling of DNA is subject to topoisomerase regulation (gyrase regulating positive supercoiling and toposiomerase regulating negative supercoiling). We suppose that the coordinates of the supercoiling are centered around the homeostatic setpoint $\sigma^* = 0.095$, coinciding with roughly 10.5 basepairs per turn in standard B-form DNA. Thus, when we write the translated state variable $\sigma = 0$ in this letter, it will correspond to the absolute, supercoiling density of gene X having average density $\sigma_X = \sigma^*$ over $n_X + L_t$ basepairs, where L_t is the basepair distance that displaced supercoils have to dissipate before confronting a topological barrier (a DNA binding protein, another gene, etc.). Throughout this letter, we will model exclusively σ_X to represent local supercoiling density—to avoid cluttered notation with multiple subscripts when talking about equilibria σ_e and reference points σ_r, we will simply denote $\sigma_X = \sigma$.

The purpose of tracking the supercoiling density is to postulate a new class of simple, transcription-supercoiling coupled models that mirror the experimentally observed, nonlinear relationships between supercoiling and transcription [15], [21], [27], [28]. For now, we focus on a single gene surrounded by a topological barrier that limits supercoiling. In particular, we consider a rate-varying chemical reaction network model, one where catalytic rates of production are dependent on the current supercoiling state σ:

$$\emptyset \xrightarrow{\mathrm{P_0 c_X k_{cat}}(\sigma)} \mathrm{m_x} \tag{1}$$

$$\mathrm{m_X} \xrightarrow{\delta_m} \emptyset \tag{2}$$

$$\sigma \xrightarrow{\delta_T \mathrm{T_0 c_X}} \sigma + 1 \tag{3}$$

$$\sigma \xrightarrow{\gamma \mathrm{G_0 c_X}} \sigma - 1 \tag{4}$$

$$\emptyset \xrightarrow{\alpha_2 \mathrm{k_{cat}}(\sigma)} \sigma, \tag{5}$$

where P_0 is the concentration of RNA polymerase, c_X is the intracellular concentration of gene X, δ_m is the rate of degradation, δ_T is the rate of Topoisomerase I cleavage, T_0 and G_0 are the intracellular concentrations of Topoisomerase I and gyrase, m_X is the concentration of mRNA for gene X, σ is the supercoiling density of the DNA spanning gene X and downstream until the next topological barrier, α_2 is an effective rate constant of positive supercoiling accumulation in σ from transcription, and $k_{\mathrm{cat}}(\sigma)$ models the supercoiling-dependent rate of transcription.

The rate of transcription $k_{\mathrm{cat}}(\sigma)$ is dependent on supercoiling [15], [21], [27], [28], [29]. In particular, we assume it follows the functional form of a radial basis function (matching empirical observations in [30], [31]:

$$k_{cat,X}(\sigma) = \beta e^{\frac{-\sigma^2}{s}}. \tag{6}$$

A derivation for the expression for α_2 is beyond the scope of this letter [15]. For now, it suffices to assume that

$$\alpha_2 = \delta_T T_0 \frac{n_X}{2h_0 L_t} \beta P_0 c_X,$$

which broadly speaking contains the conversion factor $\frac{n_X}{2h_0 L_t}$ converting twist displaced by the transcriptional flux represented by $\beta k_{cat}(\sigma) P_0 c_X$, and subsequently catalyzed locally into positive supercoiling at rate $\delta_T T_0$. The parameter h_0 estimates the number of basepairs melted during an average transcription bubble and while L_t is the basepair distance that displaced supercoils have to dissipate before confronting a topological barrier (a DNA binding protein, another gene, etc.). Effectively, this term is an approximation of the positive supercoils that result from transcriptional displacement of natural twist in the genetic region modeled by σ. A more detailed, spatial, biophysical model will follow in later work.

In total, there are five reactions that model mRNA and supercoiling dynamics jointly, two of which depend on the constants k_{cat} and α to be described below. There are two reactions to model the birth and death dynamics of mRNA. The first reaction models the transcription of mRNA m_X. It assumes polymerase concentration is vastly abundant and

that gene copy number in the cell is low enough to never approach the saturation regime of a Michaelis-Menten approximation (see Assumption 1). The second reaction models the degradation of mRNA as a first order reaction, which equates to an assumption that ribonucleases are unsaturated by their degradation substrates.

There are three reactions to model the regulation and fluctuation of supercoiling dynamics. The first reaction documents the action of a topoisomerase (TopoI) that corrects excessive negative supercoiling by introducing one positive supercoil (a right-handed turn). The second reaction describes the countering effect of a distinct topoisomerase species (gyrase) that introduces a negative supercoil to correct excessive positive supercoiling. In both of these reactions, we assume the topoisomerase and gyrases proceed in their reactions with a low, basal rate as estimated empirically from enzymatic experiments. The third reaction models increase in positive supercoiling when the transcription process displaces natural twist in gene X to downstream areas of DNA as positive supercoiling [15], [21].

Under the above assumptions, combining these five chemical reactions, with the law of mass action yields the following, simplified single-gene transcription-supercoiling model:

$$\begin{aligned}\dot{m}_X &= k_{\text{cat}}(\sigma)P_0c_X - \delta_m m_X \\ \dot{\sigma} &= \delta_T T_0 \frac{n_X}{2h_0 L_t} k_{\text{cat}}(\sigma)P_0c_X - (\gamma G_0 - \delta_T T_0)c_X\sigma. \end{aligned} \quad (7)$$

Now suppressing all subscript notation for gene X, then the abstraction for this system is of the form

$$\begin{aligned}\dot{m} &= \alpha_1 e^{-\sigma^2/s} - \delta_m m \\ \dot{\sigma} &= \alpha_2 e^{-\sigma^2/s} - (\alpha_4 - \alpha_3)\sigma. \end{aligned} \quad (8)$$

where

$$\begin{aligned}&\alpha_1 = P_0c_X\beta, \alpha_2 = \delta_T T_0 \frac{n_X}{2h_0 L_t}\beta P_0 c_X, \\ &\alpha_3 = \delta_T T_0 c_X, \alpha_4 = \gamma G_0 c_X.\end{aligned}$$

Proposition 1: When $\alpha_4 - \alpha_3 > 0$, the system (8) has a single, positive equilibrium point (m_e, σ_e) with

$$\sigma_e > 0, \quad m_e = \frac{\alpha_1}{\delta_m} e^{-\sigma_e^2/s} > 0.$$

Proof: The result follows from setting the derivative of σ above equal to 0 and noting that an origin-centered radial basis function and a positively sloped line intersecting the x (or σ) axis at the origin only cross once on the right hand side of the y-axis with $\sigma_e < 0$. Solving the first equation yields the expression for m_e. ∎

With this simplified model, we can derive the following two results about the stability of σ about the origin. These mathematical results are corroborated by prior experimental studies of the phenomenon of transcriptional bursting, which consistently shows the stability and pulsatile properties of mRNA expression [15], [22].

Theorem 1 (Lyapunov Stability): Suppose that the coupled transcription-supercoiling model (8) is written as

$$\begin{aligned}\dot{m} &= \alpha_1 e^{-(\sigma)^2/s} - \delta_m m \\ \dot{\sigma} &= \alpha_2 e^{-(\sigma)^2/s} - (\alpha_4 - \alpha_3)(\sigma).\end{aligned}$$

with simplified constants $\alpha_1, \alpha_2, \alpha_3, \alpha_4, s > 0$, where s is an empirically fitted shape coefficient of the radial basis function defined in (6). Then $(m_e, \sigma_e)^T$ is a globally, exponentially stable equilibrium point when $\alpha_4 - \alpha_3 > 0$.

Proof: The following inequalities hold due to the boundedness of radial basis functions:

$$\alpha_1 e^{-\sigma^2/s} \leq \alpha_1, \quad \alpha_2 e^{-\sigma^2/s} \leq \alpha_2$$

so we can bound the vector field by a decoupled system of the form $Ax + b$ where

$$A = \begin{bmatrix} -\delta_m & 0 \\ 0 & -(\alpha_4 - \alpha_3) \end{bmatrix}, \quad b = \begin{bmatrix} \alpha_1 \\ \alpha_2 \end{bmatrix}$$

and by the monotonicity property of integrals, since A is Hurwitz and b is time-invariant and bounded, we know that the bounding system dynamics are exponentially stable. Therefore, the original system is exponentially stable, in particular the single equilibrium point derived in Proposition 1 must be exponentially stable. ∎

Theorem 2: Given the coupled transcription-supercoiling model (8) with

$$\alpha_2 = \delta_T T_0 \frac{n_x}{2h_0 L_t}\beta P_0 c_X,$$

and $\alpha_4 - \alpha_3 > 0$ the mRNA equilibrium m_e is strictly increasing while the supercoiling equilibrium σ_e is strictly decreasing, with increasing spacing L_t between the gene and its downstream barrier.

Proof: To show strict monotonicity of the system's equilibrium point to increasing L_t we use implicit differentiation. We know that m_e and σ_e are dependent of L_t implicitly. Specifically, we know that to solve for σ_e we must solve the equation

$$0 = \delta_T T_0 \frac{n_X}{2h_0 L_t}\beta P_0 c_X e^{-\sigma_e^2/s} - (\alpha_4 - \alpha_3)\sigma_e \quad (9)$$

Multiplying by L_t gives us

$$0 = \delta_T \frac{T_0 n_X}{2h_0}\beta P c_X e^{-\sigma_e^2/s} - L_t(\alpha_4 - \alpha_3)\sigma_e \quad (10)$$

and implicitly differentiating with respect L_t yields

$$\left[\frac{\delta_T T_0 n_x \beta P_0 c_X}{h_0 s}\sigma_e e^{-\sigma_e^2/s} + L_t(\alpha_4 - \alpha_3)\right]\frac{\partial \sigma_e}{\partial L_t} = -(\alpha_4 - \alpha_3)\sigma_e$$

which solving for $\frac{\partial \sigma_e}{\partial L_t}$

$$\frac{\partial \sigma_e}{\partial L_t} = \frac{-(\alpha_4 - \alpha_3)\sigma_e}{\frac{\delta_T T_0 n_X \beta P_0 c_X \sigma_e}{h_0 s} e^{-\sigma^2/s} + L_t(\alpha_4 - \alpha_3)},$$

we see that the numerator is negative since $\sigma_e > 0$ (Proposition 1) and $-(\alpha_4 - \alpha_3) < 0$, so their product is negative. Since the denominator is always positive,

$$\frac{\partial \sigma_e}{\partial L_t} < 0.$$

Differentiating the expression for $m_e(L_t)$ we see that

$$\frac{\partial m_e(L_t)}{\partial L_t} = \frac{\alpha_1}{\delta_m} e^{-\sigma_e^2/s}\left(\frac{-2\sigma_e}{s}\frac{\partial \sigma_e}{\partial L_t}\right)$$

which is positive since $\sigma_e > 0$ and monotonically decreasing in L_t as shown above. ∎

III. Supercoiling-Transcription Model With DNA Binding Protein Interference

Now, let us consider an identical scenario as in the previous section, except now we consider the influence of a DNA binding protein that can bind to regions of DNA near the gene using a targeted binding site. For example, this may be a standard nucleoid binding protein with a specific DNA sequence binding motif or a CRISPRi based DNA binding protein that uses guide RNA targeting. Separately, one could engineer a promiscuous DNA-binding protein whose position is actuated by a magnetic control signal that is computed *in silico*. Here we investigate the theoretical ramifications of controlling $u(t) = L_t$ as a unconstrained, time-varying control signal.

The parameter L_t appears in α_2 of the simplified model (8). Here we write $u(t) = L_t$ to consider the scenario of a potentially time-varying spacer distance of a "sliding" DNA-binding protein. The experimental details of such a realization are beyond the scope of this letter, but we conceive of a scenario where a DNA-binding protein with a magnetically responsive domain is actuated by an *in silico* generated control signal from a dynamically moving magnetic field (an actuated magnetic tweezer).

Mathematically, we suppose that $u(t)$ may range from positive to negative values. A negative distance $u(t)$ would indicate that the binding protein potentially binds internally or upstream of the gene, to accumulate negative supercoiling. In this letter, we have focused primarily on positive supercoiling but a more in-depth study with both negative and positive supercoiling will be the subject of future work, pursuant to informative experimental measurements.

The revised coupled supercoiling-transcription model with input $u(t)$ takes the form

$$\begin{aligned} \dot{m} &= \alpha_1 e^{-(\sigma)^2/s} - \delta_m m \\ \dot{\sigma} &= \frac{\alpha_2}{u} e^{-(\sigma)^2/s} - (\alpha_4 - \alpha_3)\sigma. \end{aligned} \tag{11}$$

where all other α_i parameters are the same as before, but the new α_2 (excluding L_t) is given as

$$\begin{aligned} &\alpha_1 = P_0 c_X \beta, \quad \alpha_2 = \delta_T T_0 \frac{n_X}{2h_0} \beta P_0 c_X, \\ &\alpha_3 = \delta_T T_0 c_X, \quad \alpha_4 = \gamma G_0 c_X. \end{aligned}$$

Theorem 3: Given the bounded reference signals σ_r and m_r, the system (11) is asymptotically stable with the dynamic control law

$$u(t) = \frac{\alpha_2}{\alpha_1} \frac{k_2(m_r - m + \delta_m m - \dot{m}_r)}{k_1(\sigma_r - \sigma + (\alpha_4 - \alpha_3)\sigma)}, \tag{12}$$

asymptotically tracks the dynamic reference $m(t)$ and stabilizes $\sigma(t)$ about the reference σ_r, and where k_1, and k_2, define tuneable parameters to tune the convergance rates of the system.

Proof: Define the tracking error terms

$$e_\sigma = \sigma_r - \sigma, \quad e_m = m_r - m. \tag{13}$$

We will take a tiered approach in a way that utilizes the scaled, nonlinear coupling of transcription and supercoiling. Specifically, both share a radial basis function term, directly as a consequence of the physics of the phenomenon we are studying. In our model, increase in supercoiling derived from transcription is directly proportional to the rate of transcription (which is a supercoiling-dependent function). First, we will solve for the control law to stabilize $\sigma(t)$ to track the reference σ_r. Using the method of Sontag's formula [32], define the control Lyapunov function $V_\sigma(e_\sigma) = \frac{1}{2}{e_\sigma}^2$, then $\dot{V}_\sigma(e_\sigma) = e_\sigma \dot{e}_\sigma = e_\sigma(\dot{\sigma}_r - \dot{\sigma})$, and set $\dot{e}_\sigma = -k_1 e_\sigma$ to enforce negative definiteness of $\dot{V}_\sigma(e_\sigma)$ and tunability of convergence with the design parameter k_1. This guarantees that the dynamics of $e_\sigma(t)$ are asymptotically stable, which guarantees convergence of the supercoiling to a desired state. Let us now set

$$\dot{e}_\sigma = -k_1 e_\sigma = \left(\dot{\sigma}_r - \frac{\alpha_2}{u} e^{(-\sigma^2/s)} + (\alpha_4 - \alpha_3)\sigma\right) \tag{14}$$

and solving for u, we get

$$u(t) = \frac{\alpha_2 e^{-\sigma^2/s}}{k_1 e_\sigma + \dot{\sigma}_r + (\alpha_4 - \alpha_3)\sigma}. \tag{15}$$

Now we use the functional coupling relationship between supercoiling and transcription to define a control Lyapunov function

$$V_m(e_m) = \frac{1}{2} e_m^2.$$

Again, taking the derivative of

$$\begin{aligned} \dot{V}_m(e_m) &= e_m \dot{e}_m = e_m(\dot{m}_r - \dot{m}) \\ &= e_m\left(\dot{m}_r - \alpha_1 e^{-\sigma^2/s} + \delta_m m\right), \end{aligned} \tag{16}$$

and again, introducing a convergence tuning parameter for design, k_2, we obtain

$$\dot{e}_m = -k_2 e_m = \dot{m}_r - \alpha_1 e^{-\sigma^2/s} + \delta_m m, \tag{17}$$

which allows us to solve for σ in terms of $m_r(t)$ and $m(t)$. Noting the functional equivalence of elements of $u(t)$ and our current expression of $\dot{e}_m$ (a natural consequence of mRNA and supercoiling production being linked to the same transcriptional event) allowing us to write

$$e^{-\sigma^2/s} = \frac{k_2 e_m + \delta_m m - \dot{m}_r}{\alpha_1} \tag{18}$$

and so $u(t)$, the control law can be expressed as

$$\begin{aligned} u(t) &= \frac{\frac{\alpha_2}{\alpha_1}(k_2 e_m + \delta_m m - \dot{m}_r)}{k_1 e_\sigma + \dot{\sigma}_r + (\alpha_4 - \alpha_3)\sigma} \\ &= \frac{\alpha_2}{\alpha_1}\left(\frac{k_2(m_r - m) + \delta_m m - \dot{m}_r}{k_1(\sigma_r - \sigma) + \dot{\sigma}_r + (\alpha_4 - \alpha_3)\sigma}\right) \end{aligned} \tag{19}$$

Thus, we have shown, by construction, that $V_\sigma(e_\sigma)$ and $V_m(e_m)$ are control Lyapunov functions and therefore $e_\sigma(t)$ and $e_m(t)$ will converge to 0 asymptotically. ■

The mathematical form of this control law is complex, so the most feasible strategy is to compute the control law *in silico*. In *in vitro* experiments, we anticipate having direct seconds-scale measurements of $m(t)$ and high-resolution proxy measurements for $\sigma(t)$ via DNA visualization. We thus can numerically estimate the derivatives of σ and m, subject to filtering or smoothing.

TABLE I
SIMULATION PARAMETERS FOR FIG. 2, FIG. 3, AND FIG. 4

Constant	Value	Units	Reference
P_0	2.5	μM	[33]
c_x	1.0	$\$M$	n/a
σ^*	-0.5	turn/bp	n/a
s	1.0	$\text{bp}^2/\text{turns}^2$	n/a
δ_m	0.01	s^{-1}	[34]
G_0	3	μM	[35]
T_0	3	μM	[35]
γ	0.1	$\mu\text{M}^{-1}\text{s}^{-1}$	n/a
δ_T	0.05	$\mu\text{M}^{-1}\text{s}^{-1}$	n/a
h_0	0.12	μM / turn	[36]
n_X	1000	base pairs	n/a
L_t	100-1000	base pairs	n/a

IV. SIMULATION RESULTS

In this section we summarize three numerical findings with our supercoiling-transcription models from Section II and Section III: 1) we show open-loop, pulsatile response for varying spacing lengths L_t, 2) we test the dynamic control law from Theorem 3 on a sinusoidal tracking problem, and given the difficulties of dynamic control of $u(t)$, 3) we explore a switching, static control method to inform experimental design. Our simulations were conducted in Python 3.7 using the `scipy.odeint` solver, with an i7 Intel QuadCore. The code for these simulations is available upon request.

A. Pulsatile Response of the Open-Loop Supercoiling-Transcription Model

We simulated the open-loop supercoiling-transcription dynamics modeled in equation (8), the parameters are defined in Table I. In Figure 2 we simulate three different spacing lengths of $L_t = 100, 500, 1000$ basepairs. We see that $m(t)$ generates a pulsatile response, while m_e and σ_e increases monotonically with L_t, illustrating the conclusions of Theorem 2.

B. Dynamic Supercoiling Control: Control of Transcriptional Bursting

Now consider the controlled system (11), with parameters as in Table I, we simulated a dynamic supercoiling control law using the input function defined as in Theorem 3. As a theoretical challenge, we define a harmonically oscillatory reference signal: $m_r(t) = 2\sin(\frac{t}{200}) + 2.1$, with $\dot{m}_r(t) = \frac{\cos(t/200)}{100}$ is the analytical derivative of the reference signal, and $\sigma_r = 0.5$ as a static reference. The dynamics of the simulation is plotted in Figure 3. Consistent with Theorem 3, we are able to track our oscillatory reference signal over a 5000 second horizon. We see small oscillations of $\sigma(t)$ as the controller attempts to regulate $\sigma(t)$ to a constant σ_r. Here tracking is limited by the coupling of oscillatory transcriptional dynamics and supercoiling.

C. Supercoiling Control With Switching Spacer Lengths

In many cases, realizing an *in vivo* biomolecular realization of the control law in Theorem 3 may not be possible. Here we simulate the effect of a crude switching control law or bang-bang control option.

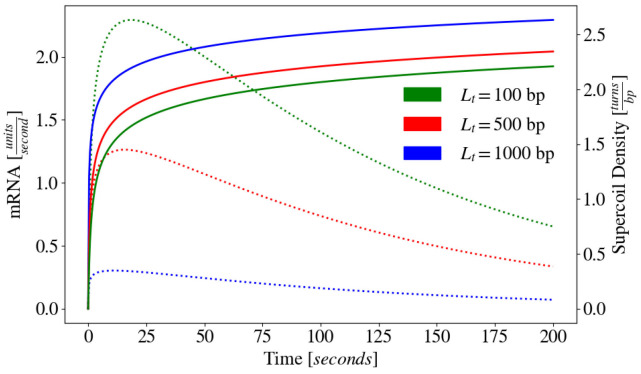

Fig. 2. The simulated response of the open loop system (8) for 3 different lengths of L_t. The solid lines represent the supercoiling density σ while the dotted lines represent the concentration of mRNA molecules m_X. With increasing coding sequence length, the amplitude of the transcriptional pulse in mRNA concentration increases.

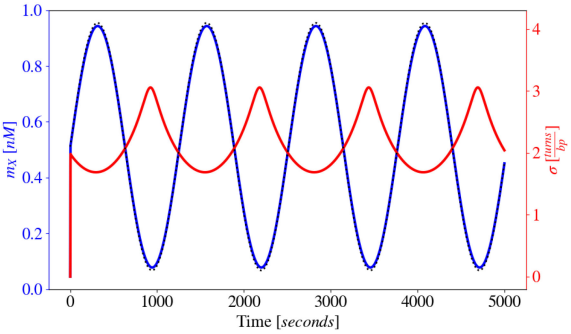

Fig. 3. Dynamic supercoiling control exhibiting transcriptional bursting. The red line depicts the supercoiling density σ while the blue line depicts the concentration of mRNA molecules m_X. The black dashed line is the reference trajectory.

Such a control law could be approximated experimentally in a variety of ways. For example, an optically sensitive protein could conditionally bind to a DNA locus, when activated by light, to achieve spacer length L_t downstream the gene of interest. In the presence of light from a different wavelength, we could cause a different protein to exclusively bind immediately behind the terminator, rendering $L_t = 0$. By alternating these signals, we can create two distinct lengths for supercoils: $n_x + L_t$ and n_x. Then $u(t)$ becomes a switching function alternating between two lengths:

$$u(t) = \begin{cases} n_x & \text{for t} \in I_1, I_3, ..I_n \\ L_t + n_x & \text{for t} \in I_2, I_4, \ldots, I_{n-1} \end{cases} \quad (20)$$

In simulation we see that the mRNA response matches a lagged version of a periodically alternating step function. The mRNA alternates between two non-zero steady-state values. The supercoiling, as expected, alternates between a positive setpoint (lower spacing) and a near-zero setpoint (ample spacing for supercoiling dissipation). The decay rate of supercoiling and mRNA states is dependent on the balance of T_0 and G_0 activity and δ_m, respectively. For illustrative purposes, the period in our simulations is set to about $p = 100$ seconds. In practice, the period would be set to allow ample

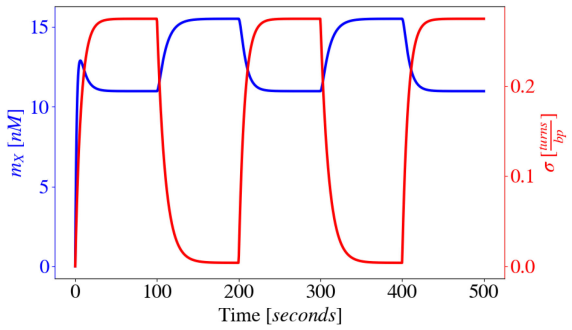

Fig. 4. Dynamic supercoiling control exhibiting transcriptional bursting. The red line depicts the supercoiling density σ while the blue line depicts the concentration of mRNA molecules m_X.

time for the rise and fall rates observed in measurements. We see that this strategy provides a coarse way to shape the frequency and duration of transcriptional bursts.

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