

Controlling mRNA Translation

Yoram Zarai, Michael Margaliot, Eduardo D. Sontag and Tamir Tuller*

Abstract

The ribosomal density along different parts of the coding regions of the mRNA molecule affect various fundamental intracellular phenomena including: protein production rates, global ribosome allocation and organismal fitness, ribosomal drop off, co-translational protein folding, mRNA degradation, and more. Thus, regulating translation in order to obtain a *desired* ribosomal profile along the mRNA molecule is an important biological problem.

We study this problem using a dynamical model for mRNA translation, called the ribosome flow model (RFM). In the RFM, the mRNA molecule is modeled as chain of n sites. The n state-variables describe the ribosomal density profile along the mRNA molecule, whereas the transition rates from each site to the next are controlled by $n + 1$ positive constants. To study the problem of controlling the density profile, we consider some or all of the transition rates as time-varying controls.

We consider the following problem: given an initial and a desired ribosomal density profile in the RFM, determine the time-varying values of the transition rates that steer the system to this density profile, if they exist. More specifically, we consider two control problems. In the first, all transition rates can be regulated and the goal is to steer the ribosomal density profile and the protein production rate from a given initial value to a desired value. In the second, a *single* transition rate is controlled and the goal is to steer the production rate to a desired value. In the first case, we show that the system is controllable, i.e. the control is powerful enough to steer the system to any desired value, and we provide simple closed-form expressions for *constant* control functions (or transition rates) that asymptotically steer the system to the desired value. In the second case, we show that we can steer the production rate to any desired value in a feasible region determined by the other constant transition rates. We discuss some of the biological implications of these results.

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Index Terms

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I. INTRODUCTION

The process in which the genetic information coded in the DNA is transformed into functional proteins is called *gene expression*. It consists of two major steps: *transcription* of the DNA code into messenger RNA (mRNA) by RNA polymerase, and *translation* of the mRNA into proteins. During the translation step, macro-molecules called ribosomes unidirectionally traverse the mRNA, decoding it codon by codon into a corresponding chain of amino-acids that is folded co-translationally and post-translationally to become a functional protein. The rate in which proteins are produced during the translation step is called the protein translation rate or protein production rate.

Translation takes place in all organisms and tissues under almost all conditions. Thus, developing a better understanding of how translation is regulated has important implications to many scientific disciplines, including medicine, evolutionary biology, and synthetic biology. Developing and analyzing computational models of translation may provide important insights on this biological process. Such models can also aid in integrating and analyzing the rapidly increasing experimental findings related to translation (see, e.g., [8], [51], [41], [6], [45], [10], [36]).

Controlling the expression of heterologous genes in a host organism in order to synthesize new proteins, or to improve certain aspects of the host fitness, is an essential challenge in biotechnology and synthetic biology [40], [29], [3], [52], [2]. Computational models of translation are particularly important in this context, as they allow simulating and analyzing the effect of various manipulations of the gene expression machinery and/or the genetic material, and can thus save considerable time and effort by guiding biologists towards promising experimental directions.

The ribosome flow along the mRNA is regulated by various translation factors (e.g., initiation and elongation factors, tRNA and Aminoacyl tRNA synthetase concentrations, and amino-acid concentrations) in order to achieve both a suitable ribosomal density profile along the mRNA, and a desired protein production rate. Indeed, it is known that the ribosomal density profile and the induced ribosome speed profile along the mRNA molecule can affect various fundamental intracellular phenomena. For example, it is known that the folding of translated proteins may take place co-translationally, and inaccurate translation

speed can contribute to protein miss-folding [12], [22], [55]. It is also known that the profile of ribosome density may affect the degradation of mRNA, ribosomal collisions, abortion and allocation, transcription, and more [12], [22], [23], [13], [55], [52], [35].

Thus, a natural question is whether it is possible, by controlling the transition rates along the mRNA, to steer the ribosome density along the mRNA molecule from any initial profile to any desired profile in finite time. In the language of control theory, the question is whether the system is *controllable* (see, e.g. [48]). Controllability of networked systems is recently attracting considerable interest (see e.g. [24]). An important problem in this context is to determine a minimal set of “driver nodes” within the network such that controlling these nodes makes the entire network controllable (see e.g. [30]).

Controllability of mRNA translation is also important in synthetic biology, e.g. in order to design *cis* or *trans* intra-cellular elements that yield a desired ribosome density profile (or to determine if such a design is possible). Another related question arises in evolutionary systems biology, namely, determine if a certain translation-related phenotype can be obtained by evolution.

Our study is also related to cancer evolution. Indeed, it is well-known that cancerous cells undergo evolution that modulates their translation regime. It has been suggested that various mutations that accumulate during tumorigenesis may affect both translation initiation [16], [25] and elongation [53], [50] of genes related to cell proliferation, metabolism, and invasion. Specifically, the results reported in the recent study [16] support the conjecture that cancerous mutations can significantly change the ribosome density profile on the mRNAs of dozen of genes.

The standard mathematical model for ribosome flow is the *totally asymmetric simple exclusion process* (TASEP) [46], [56]. In this model, particles hop unidirectionally along an ordered lattice of L sites. Every site can be either free or occupied by a particle, and a particle can only hop to a free site. This simple exclusion principle models particles that have “volume” and thus cannot overtake one other. The hops are stochastic and the rate of hopping from site i to site $i + 1$ is denoted by γ_i . A particle can hop to [from] the first [last] site of the lattice at a rate α [β]. The flow through the lattice converges to a steady-state value that depends on L and the parameters $\alpha, \gamma_1, \dots, \gamma_{L-1}, \beta$. In the context of translation, the lattice models the mRNA molecule, the particles are ribosomes, and simple exclusion means that a ribosome cannot overtake a ribosome in front of it. TASEP has become a fundamental model in non-equilibrium statistical mechanics, and has been applied to model numerous natural and artificial processes [44].

The *ribosome flow model* (RFM) [39] is a *deterministic* model for translation that can be derived via

a dynamic mean-field approximation of TASEP [44, section 4.9.7] [4, p. R345]. In the RFM, mRNA molecules are coarse-grained into n consecutive sites of codons (or groups of codons). The state variable $x_i(t) : \mathbb{R}_+ \rightarrow [0, 1]$, $i = 1, \dots, n$, describes the normalized ribosomal occupancy level (or density) of site i at time t , where $x_i(t) = 1$ [$x_i(t) = 0$] indicates that site i is completely full [empty] at time t . Thus, the vector $\begin{bmatrix} x_1(t) & \dots & x_n(t) \end{bmatrix}'$ describes the complete *ribosomal density profile* along the mRNA molecule at time t . A variable denoted $R(t)$ describes the *protein production rate* at time t . A nonnegative parameter λ_i , $i = 0, \dots, n$, controls the transition rate from site i to site $i+1$, where λ_0 [λ_n] is the initiation [exit] rate.

In order to better understand how translation is regulated, we consider the RFM with some or all of the constant transition rates replaced by time-varying control functions that take non-negative values for all time t . The idea here that we can control these functions as desired.

We consider two control problems. In the first, all the $n+1$ λ_i s are replaced by control functions and the problem is to control these functions such that the ribosomal density profile and the production rate are steered from a given initial value to a desired value. We use the term ‘‘augmented profile’’ to indicate the combination of the ribosomal density profile and the production rate. In the second control problem, a *single* λ_i is replaced by a control $u(t)$ and the problem is to control $u(t)$ such that the production rate is steered to a desired value. Note that in the first problem the $n+1$ -dimensional vector describing the augmented profile is controlled using $n+1$ control functions, and in the second problem a single variable is controlled using a scalar control.

We show that in both cases the resulting control system is *controllable*, i.e. the control is always ‘‘powerful’’ enough to steer the system from any initial state to any desired state in finite time. We also show that there always exists a control that steers the system as desired, and is the time concatenation of two controls:

$$u(t) = \begin{cases} v, & t \in [0, T - \varepsilon), \\ w(t), & t \in [T - \varepsilon, T], \end{cases} \quad (1)$$

with $\varepsilon > 0$ and very small. The constant control v is given in a *simple and explicit* expression that depends only on the desired final state. It guarantees that this state becomes the unique attracting steady-state ribosomal density and production rate of the RFM dynamics. For example, in the problem of controlling the density profile and the production rate to desired final values x^f and R^f respectively (‘‘ f ’’ for final),

the solution of the controlled RFM for *any* initial condition $x(0)$ and $R(0)$ satisfies

$$\begin{aligned}\lim_{t \rightarrow \infty} x(t) &= x^f, \\ \lim_{t \rightarrow \infty} R(t) &= R^f.\end{aligned}\tag{2}$$

This means that for all practical reasons, one may simply apply the constant control $u(t) \equiv v$ for all $t \geq 0$. Note that (2) means that the exact values of $x(0)$ and $R(0)$, i.e. the initial values of the density profile and production rate, are actually not needed. This is important, as accurately measuring $x(0)$ and $R(0)$ in practice may be difficult. The control $w(t)$ in (1) is needed only to guarantee that $x(T) = x^f$ and $R(T) = R^f$ for some *finite* time T . The existence of such a $w(t)$ follows from standard accessibility arguments, but $w(t)$ is not given explicitly.

Different aspects of translation regulation, usually under natural conditions, have been studied before (see, for example, [17]). There are also a few studies on experimental and computational heuristics for mRNA translation engineering and optimization (see, for example, [43], [49]), and studies related to the way translation regulation is encoded in the transcript (e.g. [57], [32]). However, to the best of our knowledge, this is the first study on controllability of translation in a realistic model for translation. Also, previous studies on translation optimization only considered protein levels or production rate (e.g. [43]), but not the problem of controlling the entire profile of mRNA decoding rates as is done here.

The remainder of this paper is organized as follows. The following section provides a brief overview of the RFM and its generalizations into a control system. In order to make this paper accessible to a larger audience, Appendix A provides a very brief review of controllability, while demonstrating some of the concepts using the RFM. Section III presents our main results on the controlled RFM. We also discuss the biological ramifications of our results. To streamline the presentation, all the proofs are placed in Appendix B.

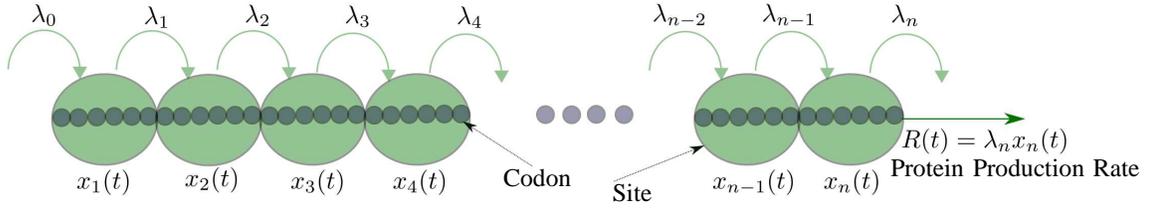


Fig. 1. The RFM models a chain of n sites of codons (or groups of codons). The state variable $x_i(t) \in [0, 1]$ represents the normalized ribosome occupancy of site i at time t . The elongation rate from site i to site $i + 1$ is λ_i , with λ_0 [λ_n] denoting the initiation [exit] rate. The production rate at time t is $R(t) = \lambda_n x_n(t)$.

II. RIBOSOME FLOW MODEL

In this section, we quickly review the RFM and describe its generalizations into a control system. The dynamics of the RFM with n sites is given by n non-linear first-order ordinary differential equations:

$$\begin{aligned}
 \dot{x}_1 &= \lambda_0(1 - x_1) - \lambda_1 x_1(1 - x_2), \\
 \dot{x}_2 &= \lambda_1 x_1(1 - x_2) - \lambda_2 x_2(1 - x_3), \\
 \dot{x}_3 &= \lambda_2 x_2(1 - x_3) - \lambda_3 x_3(1 - x_4), \\
 &\vdots \\
 \dot{x}_{n-1} &= \lambda_{n-2} x_{n-2}(1 - x_{n-1}) - \lambda_{n-1} x_{n-1}(1 - x_n), \\
 \dot{x}_n &= \lambda_{n-1} x_{n-1}(1 - x_n) - \lambda_n x_n.
 \end{aligned} \tag{3}$$

If we define $x_0(t) := 1$ and $x_{n+1}(t) := 0$ then (3) can be written more succinctly as

$$\dot{x}_i = \lambda_{i-1} x_{i-1}(1 - x_i) - \lambda_i x_i(1 - x_{i+1}), \quad i = 1, \dots, n. \tag{4}$$

Recall that the state variable $x_i(t) : \mathbb{R}_+ \rightarrow [0, 1]$ describes the normalized ribosomal occupancy level (or density) of site i at time t , where $x_i(t) = 1$ [$x_i(t) = 0$] indicates that site i is completely full [empty] at time t . Eq. (4) can be explained as follows. The flow of ribosomes from site i to site $i + 1$ is $\lambda_i x_i(t)(1 - x_{i+1}(t))$. This flow is proportional to $x_i(t)$, i.e. it increases with the occupancy level at site i , and to $(1 - x_{i+1}(t))$, i.e. it decreases as site $i + 1$ becomes fuller. This corresponds to a “soft” version of the simple exclusion principle in TASEP. Note that the maximal possible flow from site i to site $i + 1$ is the transition rate λ_i . Eq. (4) thus states that the time derivative of state-variable x_i is the flow entering site i from site $i - 1$, minus the flow exiting site i to site $i + 1$.

The ribosome exit rate from site n at time t is equal to the protein production rate at time t , and is denoted by $R(t) := \lambda_n x_n(t)$ (see Fig. 1). Note that x_i is dimensionless, and every rate λ_i has units

of 1/time.

A system where each state variable describes the amount of “material” in some compartment, and the dynamics describes the flow of material within the compartments and also with the surrounding environment is called a *compartmental system* [19]. Compartmental systems proved to be useful models in various biological domains including physiology, pharmacokinetics, population dynamics, and epidemiology [5], [15], [18]. The RFM is clearly a nonlinear compartmental system, with x_i denoting the normalized amount of “material” in compartment i , and the flow follows a “soft” simple exclusion principle. The controllability of *linear* compartmental system has been addressed in several papers [20], [14].

Let $x(t, a)$ denote the solution of (3) at time $t \geq 0$ for the initial condition $x(0) = a$. Since the state-variables correspond to normalized occupancy levels, we always assume that a belongs to the closed n -dimensional unit cube:

$$C^n := \{x \in \mathbb{R}^n : x_i \in [0, 1], i = 1, \dots, n\}.$$

It has been shown in [27] that if $a \in C^n$ then $x(t, a) \in C^n$ for all $t \geq 0$, that is, C^n is an invariant set of the dynamics. Let $\text{int}(C^n)$ denote the interior of C^n , and let ∂C^n denote the boundary of C^n . Ref. [27] has also shown that the RFM is a *tridiagonal cooperative dynamical system* [47], and that (3) admits a *unique* steady-state point $e(\lambda_0, \dots, \lambda_n) \in \text{int}(C^n)$ that is globally asymptotically stable, that is, $\lim_{t \rightarrow \infty} x(t, a) = e$ for all $a \in C^n$ (see also [26]). This means that the ribosome density profile always converges to a steady-state profile that depends on the rates, but not on the initial condition. In particular, the production rate $R(t) = \lambda_n x_n(t)$ converges to a steady-state value:

$$R := \lambda_n e_n. \tag{5}$$

At steady-state (i.e, for $x = e$), the left-hand side of all the equations in (3) is zero, so

$$\begin{aligned} \lambda_0(1 - e_1) &= \lambda_1 e_1(1 - e_2) \\ &= \lambda_2 e_2(1 - e_3) \\ &\vdots \\ &= \lambda_{n-1} e_{n-1}(1 - e_n) \\ &= \lambda_n e_n \\ &= R. \end{aligned} \tag{6}$$

This yields

$$R = \lambda_i e_i (1 - e_{i+1}), \quad i = 0, \dots, n, \quad (7)$$

where $e_0 := 1$ and $e_{n+1} := 0$.

Remark 1 *One may view (6) as a mapping from the rates $[\lambda_0, \dots, \lambda_n]'$ to the steady-state density profile and production rate $[e_1 \dots e_n \ R]'$. For the purposes of this paper, it is important to note that this mapping is invertible. Indeed, Eq. (7) implies that given a desired steady-state density profile and production rate $[e_1 \dots e_n \ R]' \in (0, 1)^n \times \mathbb{R}_{++}$ one can immediately determine the transition rates that yield this profile, namely,*

$$\lambda_i = \frac{R}{e_i (1 - e_{i+1})}, \quad i = 0, \dots, n, \quad (8)$$

where $e_0 := 1$, and $e_{n+1} := 0$.

Note that (8) implies that λ_i increases with R and e_{i+1} , and decreases with e_i . This is intuitive, as a larger λ_i implies larger rate of ribosomes flow from site i to site $i + 1$, as well as an increase in the steady-state production rate [33]. Thus, given a desired profile with larger R and e_{i+1} , and a smaller e_i , the required transition rates include a larger value for λ_i .

From a biophysical point of view, this means that if there are no constraints on the transition rates then we can engineer any desired density profile together with a desired production rate. This is not trivial since in the RFM (and any other reasonable translation model) each of the state variables is affected by *all* the transition rates. In addition to applications in functional genomics and molecular evolution, the observation in Remark 1 is also related to problems in synthetic biology where the goal is to re-engineer the mRNA molecule so as to obtain a desired density profile and production rate (see Fig. 2).

For more on the analysis of the RFM using tools from systems and control theory, see [54], [33], [34], [28], [38]. The RFM models translation on a single isolated mRNA molecule. A network of RFMs, interconnected through a common pool of “free” ribosomes has been used to model simultaneous translation of several mRNA molecules while competing for the available ribosomes [37] (see also [1] for some related ideas).

In this paper, we analyze the regulation of translation using the RFM. To do this, we first introduce two generalizations of the RFM into a control system.

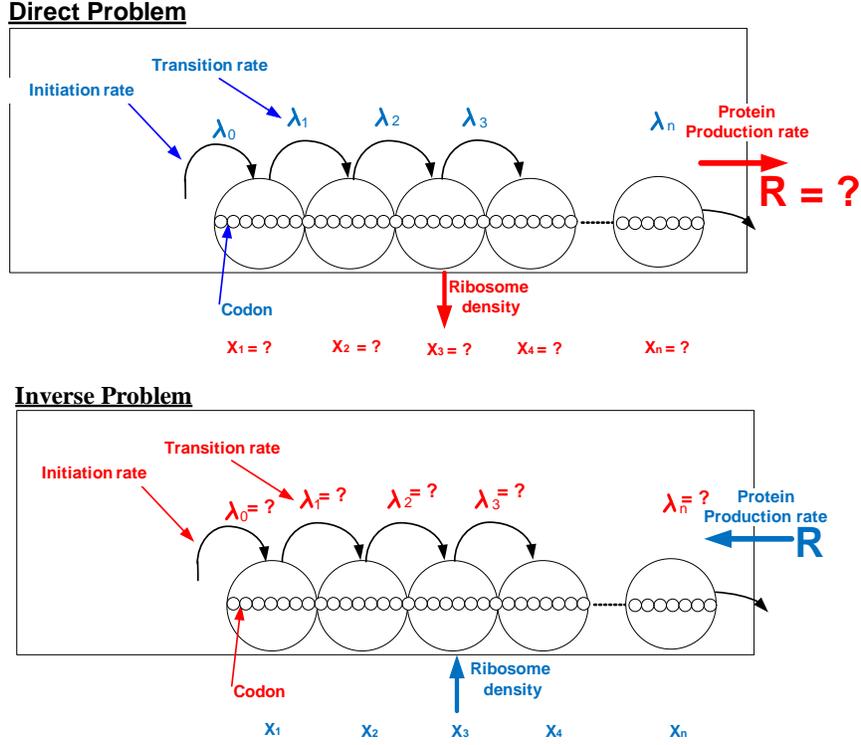


Fig. 2. Upper part: Previous studies considered the direct problem: given the RFM parameters, i.e. the set of transition rates, λ_i , analyze the dynamics of the RFM ribosome densities x_i , and the production rate R . Lower part: here we consider the inverse problem: given a desired profile of ribosomal densities $x_i, i = 1, \dots, n$, and a desired production rate R , find the set of transition rates λ_i that steer the dynamics to this profile.

A. The Controlled RFM

1) *State- and Output-Controllability:* Assume that all the rates $\lambda_0, \dots, \lambda_n$ can be controlled. Thus, we replace every λ_i in the RFM by a function $u_i(t) : \mathbb{R}_+ \rightarrow \mathbb{R}_+$. The set of admissible controls \mathbb{U} includes all the functions that are measurable, bounded and take non-negative values for all $t \geq 0$. In the context of translation, controlling the $u_i(t)$ s corresponds to dynamically varying translation factors that regulate the initiation, elongation, and exit rates along the mRNA molecule.

The problem we consider is whether it is possible, using the $n + 1$ control functions, to steer x and R from any initial condition to any desired conditions $x^f \in \text{int}(C^n)$ and $R^f \in \mathbb{R}_{++}$ in finite time.

Of course, independently controlling all the transition rates may be difficult to do in practice, so we also consider another controlled version of the RFM.

B. Output Controllability

Pick $i \in \{0, \dots, n\}$. Replace the single rate $\lambda_i(t)$ by a scalar non-negative control function $u(t)$. This models the case where a single transition rate can be controlled. In this case, we are interested in using $u(t)$

to steer only the production rate R to a desired value R^f in finite time.

The problem we consider is whether it is possible to steer R from any initial condition to any feasible value. Of course, the set of feasible values is determined by the other, fixed, transition rates.

We show that both control problems described above are *controllable*. In other words, the control authority is always powerful enough to obtain any feasible desired density profile and/or production rate. This is a primarily theoretical result. However, we also show that there exist *constant* controls that asymptotically steer the controlled RFM to the desired densities/production rate. In the problem of controlling all the rates, these constant values are given in a simple and *closed-form* expression. In the second control problem, this constant value can be easily found numerically using a simple line search.

Our results are biologically relevant in several important applications. First, the problem we study, namely controlling the entire ribosomal profile using the transition rates, seems to be a fundamental problem in synthetic biology, and our results suggest that it can be addressed using a combination of mathematical, computational, and experimental approaches. Our results also provide an initial but explicit solution to this problem. While the model and problems may be relatively simple, they may provide a reasonable approximation to the biological solution in some cases. They may also be used as a starting point for addressing and solving similar problems in more comprehensive models of translation.

The next section describes our main results. Readers who are not familiar with controllability analysis may consult Appendix A for a quick review of this topic.

III. MAIN RESULTS

As noted above, we consider two control problems for the RFM. We now describe their exact mathematical formulation.

A. State- and Output-Controllability

Let $\Omega := C^n \times \mathbb{R}_+$. Assume first that all the $n + 1$ transition rates can be controlled. The control is then $u(t) = [u_0(t), \dots, u_n(t)]'$ and the dynamics of the controlled RFM with output $R(t)$ is described by:

$$\begin{aligned} \dot{x}_i(t) &= u_{i-1}(t)x_{i-1}(t)(1 - x_i(t)) - u_i(t)x_i(t)(1 - x_{i+1}(t)), \quad i = 1, \dots, n, \\ R(t) &= u_n(t)x_n(t). \end{aligned} \tag{9}$$

We define the admissible set \mathbb{U} as the set of measurable and bounded controls taking values in \mathbb{R}_+^{n+1} for all time t .

Problem 1 *Given arbitrary $x^s, x^f \in \text{int}(C^n)$ and $R^s, R^f \in \mathbb{R}_{++}$, does there always exist a time $T \geq 0$ and a control $u \in \mathbb{U}$ such that $x(T, u, x^s) = x^f$ and $R(T, u, R^s) = R^f$?*

Our first main result shows that the answer to this question is yes.

Theorem 1 *The controlled RFM (9) is state- and output-controllable on $\text{int}(\Omega)$.*

This means that the control is “powerful” enough to steer the system, in finite time, from any initial augmented profile to any desired final.

However, Theorem 1 is mainly of theoretical interest, as it does not entail a description of the required control. The next result provides a simple closed-form solution for a control that *asymptotically* steers the system to x^f and R^f from any initial condition. In other words, it practically solves the *control synthesis problem*.

Proposition 1 *Fix an arbitrary $x^f = [x_1^f \ \dots \ x_n^f]' \in \text{int}(C^n)$ and $R^f \in \mathbb{R}_{++}$. Define $v \in \mathbb{R}_{++}^{n+1}$ by*

$$v_i := \frac{R^f}{x_i^f(1 - x_{i+1}^f)}, \quad i = 0, \dots, n, \quad (10)$$

where $x_0^f := 1$ and $x_{n+1}^f := 0$. Then for any $x^s \in C^n$ and any $R^s \in \mathbb{R}_+$ applying the constant control $u(t) \equiv v$ in (9) yields

$$\lim_{t \rightarrow \infty} x(t, u, x^s) = x^f, \quad \lim_{t \rightarrow \infty} R(t, u, R^s) = R^f. \quad (11)$$

This means that the controlled RFM (9) is asymptotically state- and output-controllable on $\text{int}(\Omega)$. An important property of v is that it does not depend on x^s nor R^s , but only on the desired profile x^f and R^f . This is important as measuring x^s , that is, the initial ribosomal profile along the mRNA, may be difficult due to the current limitations in measuring ribosome densities (see, for example, [7], [9], [11]).

The proof of Proposition 1 follows immediately from Remark 1. Indeed, using the constant control $u(t) \equiv v$ amounts to setting the desired density profile x^f as the steady-state densities of the dynamics, and R^f as the steady-state production rate. Since this steady-state is globally asymptotically stable on $\text{int}(\Omega)$, this implies (11). ■

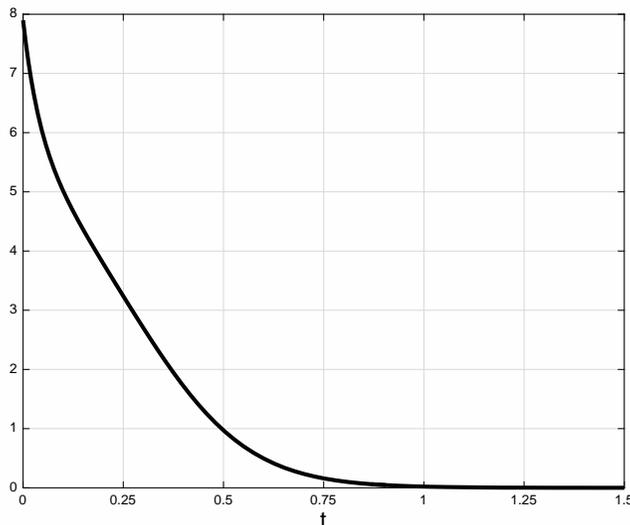


Fig. 3. The error $|x(t) - x^f|_1 + |R(t) - R^f|_1$ as a function of t .

It is important to emphasize the difference between Theorem 1 and Proposition 1. The first result guarantees the ability of steering the density profile and production rate to the desired values in *finite time*, but does not provide an explicit control. Proposition 1 provides an explicit, closed-form expression for fixed controls that achieve the desired goal, but only asymptotically.

Example 1 Consider the controlled RFM with dimension $n = 5$. Suppose that we would like to steer the ribosomal density profile along the mRNA molecule to $[0.8 \ 0.1 \ 0.1 \ 0.1 \ 0.1]'$, and the production rate to 1.5. The profile here is motivated by the fact that low ribosome abundance after the ORF reduces ribosome “traffic jams” that may lead to ribosome drop off. Setting $x^f = [0.8 \ 0.1 \ 0.1 \ 0.1 \ 0.1]'$, $R^f = 1.5$, and applying (10) yields

$$v = [30/4 \ 25/12 \ 50/3 \ 50/3 \ 50/3 \ 15]'$$

Fig. 3 depicts the error $|x(t, u, x^s) - x^f|_1 + |R(t, u, R^s) - R^f|_1$ (where $|z|_1$ denotes the L_1 norm of the vector z) for the initial conditions $x^s = [0.5 \ 0.5 \ 0.5 \ 0.5 \ 0.5]'$, $R^s = 0.5$, and the control $u(t) \equiv v$. It may be observed that the error decays at an exponential rate to zero. Thus, this control steers the system arbitrarily close to the desired final density profile x^f and production rate R^f . \square

Example 1 suggests that even though Proposition 1 provides only an asymptotic solution, the given fixed controls provide a good practical solution.

B. Output Controllability

Pick $i \in \{0, 1, \dots, n\}$ and $c > 0$. Fix arbitrary values for the rates $\lambda_j > 0$, $j \neq i$, and assume that we can control the single transition rate λ_i within the range $[0, c]$. This means that λ_i is replaced by a time-varying control u_i , and the set of admissible controls \mathbb{U} is the set of measurable scalar functions taking values in $[0, c]$ for all $t \geq 0$. This problem represents a more realistic scenario, as we do not assume that we can control all the translation rates along the mRNA, and also the allowed control action is bounded by the value c .

In this case, we are controlling a single variable and thus we do not aim to regulate the entire ribosomal density, but only the production rate $R(t)$, i.e. we are regulating the output. Of course, not every value of $R(t)$ is possible, because of the other, fixed transition rates. One can in principle define the reachable set of $R(t)$ based on the fact that the state trajectories evolve on C^n . For example, if $i \neq n$ then $R(t) = \lambda_n x_n(t)$ implies that one can define the reachable set as $[0, \lambda_n]$. However, this definition is not really relevant. Indeed, assume that some rate λ_k is much smaller than all the other rates and also much smaller than c . Then regardless of the specific control used it is clear that after some time $R(t)$ will also be small, as λ_k will be the limiting factor, and so after some time it will become impossible to steer the production rate to every desired value in the set $[0, \lambda_n]$.

We define a more meaningful reachable set for the production rate as follows. For every time $T \geq 0$ and every initial condition $x_0 \in C^n$, let $\Omega^i(T, x_0) \subset \mathbb{R}_+$ denote the set of production rates that can be attained at some time $t \geq T$ with $x(0) = x_0$. Define the *large-time reachable set* of R as

$$\Omega^i(x_0) := \bigcap_{T \geq 0} \Omega^i(T, x_0).$$

Although the RFM is a nonlinear model, this set can be characterized explicitly.

Proposition 2 For $\ell_0, \dots, \ell_n > 0$ define a $(n+2) \times (n+2)$ symmetric, tridiagonal, and componentwise

nonnegative matrix $A = A(\ell_0, \dots, \ell_n)$ by

$$A := \begin{bmatrix} 0 & \ell_0^{-1/2} & 0 & 0 & \dots & 0 & 0 \\ \ell_0^{-1/2} & 0 & \ell_1^{-1/2} & 0 & \dots & 0 & 0 \\ 0 & \ell_1^{-1/2} & 0 & \ell_2^{-1/2} & \dots & 0 & 0 \\ & & & \vdots & & & \\ 0 & 0 & 0 & \dots & \ell_{n-1}^{-1/2} & 0 & \ell_n^{-1/2} \\ 0 & 0 & 0 & \dots & 0 & \ell_n^{-1/2} & 0 \end{bmatrix}. \quad (12)$$

Let $\zeta_{MAX}(A)$ denote the maximal eigenvalue of A .¹ Then for any $x_0 \in C^n$,

$$\Omega^i = [0, M], \quad (13)$$

where $M := (\zeta_{MAX}(A(\lambda_0, \dots, \lambda_{i-1}, c, \lambda_{i+1}, \dots, \lambda_n)))^{-2}$.

Note that (13) implies that Ω^i does not depend on x_0 , but only on the parameters λ_j , $j \neq i$, and the value c .

Remark 2 Consider the case $c \rightarrow \infty$. Then $c^{-1/2} \rightarrow 0$, so the largest eigenvalue of $A(\lambda_0, \dots, \lambda_{i-1}, c, \lambda_{i+1}, \dots, \lambda_n)$ tends to

$$\max\{\zeta_{MAX}(A(\lambda_0, \dots, \lambda_{i-1})), \zeta_{MAX}(A(\lambda_{i+1}, \dots, \lambda_n))\},$$

so

$$M = \min\{(\zeta_{MAX}(A(\lambda_0, \dots, \lambda_{i-1})))^{-2}, (\zeta_{MAX}(A(\lambda_{i+1}, \dots, \lambda_n)))^{-2}\}. \quad (14)$$

In other words, when the transition rate from site i to site $i + 1$ goes to infinity the maximal possible steady-state production rate will be the minimum of two steady-state production rates: the first [second] in an RFM of length $i - 1$ [$n - i - 1$] and parameters $\lambda_0, \dots, \lambda_{i-1}$ [$\lambda_{i+1}, \dots, \lambda_n$]. This demonstrates how in this case the other, fixed rates, being the limiting factors, determine the feasible set for the production rate.

From the biological point of view this means that if the transition rate along some region of the mRNA is very high (and thus not rate limiting) the production rate will depend only on the transition rates before and after this region, as these include the rate limiting factor. Also, the large-time reachable set for the

¹It is clear that the eigenvalues are real as A is symmetric. Since A is also nonnegative and irreducible the eigenvalues are distinct.

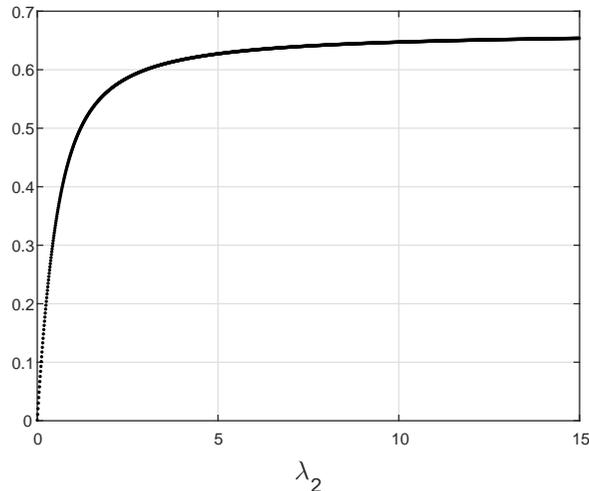


Fig. 4. Maximal steady-state production rate $(\zeta_{MAX}(A(1, 2, \lambda_2, 3)))^{-2}$ for $\lambda_2 \in [0, 15]$.

production rate will be constrained by the rate limiting transition rates.

Example 2 Consider a controlled RFM with length $n = 3$, rates

$$\lambda_0 = 1, \lambda_1 = 2, \lambda_3 = 3, \quad (15)$$

and control $u(t) = \lambda_2(t)$. Suppose that the admissible set \mathbb{U} is the set of functions taking values in $[0, c]$, with $c = 15$. Fig. 4 depicts $(\zeta_{MAX}(A(1, 2, \lambda_2, 3)))^{-2}$, for $\lambda_2 \in [0, 15]$. It may be seen that this is a strictly increasing function of λ_2 . A calculation yields (all numbers are to four digit accuracy) $(\zeta_{MAX}(A(1, 2, 15, 3)))^{-2} = 0.6538$, so $\Omega^2 = [0, 0.6538]$.

Note that if we take $c \rightarrow \infty$ then (14) yields

$$\begin{aligned} \min\left\{\left(\zeta_{MAX}\left(\begin{bmatrix} 0 & 1 & 0 \\ 1 & 0 & 2^{-1/2} \\ 0 & 2^{-1/2} & 0 \end{bmatrix}\right)\right)^{-2}, \left(\zeta_{MAX}\left(\begin{bmatrix} 0 & 3^{-1/2} \\ 3^{-1/2} & 0 \end{bmatrix}\right)\right)^{-2}\right\} &= \min\{2/3, 3\} \\ &= 2/3. \end{aligned}$$

□

The next result analyzes the output controllability in Ω^i .

Proposition 3 *The controlled RFM with λ_i replaced by a control $u(t)$ is output-controllable in $\text{int}(\Omega^i)$.*

This means that regulating a single transition rate is still “powerful” enough to steer the production rate from any initial value to any desired final value $R^f \in \text{int}(\Omega^i)$ in finite time.

It follows from the proof of Proposition 2 (see Appendix B) that the controlled RFM is asymptotically controllable in Ω^i , even when \mathbb{U} is restricted to constant controls only. Furthermore, since $\zeta_{MAX}(A(\ell_0, \dots, \ell_n))$ is a strictly decreasing function of every ℓ_i , finding the constant value c that asymptotically steers the system to a desired value $R^f \in \text{int}(\Omega)$ can be easily solved numerically using a simple line search. The next example demonstrates this.

Example 3 Consider again the controlled RFM in Example 2. Recall that the admissible set \mathbb{U} is the set of functions taking values in $[0, c]$, with $c = 15$. Assume that our goal is to asymptotically steer the production rate to, say, $R^f = 1/2$. A simple line search shows that the corresponding value of λ_2 should be $\lambda_2 \approx 1.2$ (in this particular case this can also be verified analytically using (14)). Indeed, (6) implies that for the rates in (15) and $\lambda_2 = 1.2$ the equilibrium point is $e = [1/2 \quad 1/2 \quad 1/6]'$, so $R = \lambda_3 e_3 = 1/2$. Thus, the control $u(t) \equiv 1.2$ asymptotically steers the system output to $R = 1/2$. \square

C. Sensitivity analysis

In practice, the applied controls are never exactly equal to the desired values and therefore it is important to understand the effect of small perturbations in the control values on the desired augmented profile. Since we are basically considering constant controls, it is enough to study the sensitivity of the steady-state density profile of the RFM to small changes in the λ_i 's. The sensitivity of the steady-state production rate R with respect to the λ_i s has been studied in [34].

Proposition 4 Consider the RFM with dimension n , and let $e := [e_1 \quad \dots \quad e_n]'$ denote the corresponding equilibrium point in $\text{int}(C^n)$. Pick $i \in \{0, \dots, n\}$. Then $\frac{\partial}{\partial \lambda_i} e_k$ exists for all k , and

$$\begin{aligned} \frac{\partial}{\partial \lambda_i} e_k &< 0, \quad \text{for all } k \leq i, \\ \frac{\partial}{\partial \lambda_i} e_k &> 0, \quad \text{for all } k > i. \end{aligned} \tag{16}$$

Thus, increasing λ_i decreases [increases] the steady-state densities in sites $1, \dots, i$ [sites $i + 1, \dots, n$]. This is reasonable, as increasing λ_i increases the transition rate from site i to site $i + 1$ (see also [37] for some related considerations).

Example 4 Recall from Example 1 that for the RFM with $n = 5$ the control

$$u(t) \equiv \left[30/4 \quad 25/12 \quad 50/3 \quad 50/3 \quad 50/3 \quad 15 \right]',$$

yields the steady-state augmented profile:

$$\left[e \quad R \right]' = \left[0.8 \quad 0.1 \quad 0.1 \quad 0.1 \quad 0.1 \quad 1.5 \right]'. \quad (17)$$

Let $\tilde{u}(t) \equiv \left[30/4 \quad 25/12 \quad (50/3) + \varepsilon \quad 50/3 \quad 50/3 \quad 15 \right]'$, with $\varepsilon := 0.2$ i.e. the same transition rates as before, but with ε added to λ_2 . Using (6) shows that \tilde{u} yields the steady-state augmented profile

$$\left[\tilde{e} \quad \tilde{R} \right]' = \left[0.7998 \quad 0.0989 \quad 0.1001 \quad 0.1001 \quad 0.1001 \quad 1.5013 \right]'$$

(all numbers are to four digit accuracy). Comparing this to (17) shows that the steady-state values at sites 1, 2 decreased, and those at sites 3, 4, 5 increased. \square

IV. DISCUSSION

Regulating the ribosomal density profile along the mRNA molecule, and not only the protein production rate, is an important problem in evolutionary biology, biotechnology, and synthetic biology because this density profile affects various fundamental intracellular processes including mRNA degradation, protein folding, ribosomal allocation and abortion, and more (see, for example, [55], [23], [13], [21], [31], [52]). It seems that there are still considerable gaps in our understanding of how the density profile is regulated, and how it can be re-engineered. In this paper, we addressed this issue by analyzing a mathematical model for ribosome flow, the RFM, using tools from systems and control theory.

Our results indicate that if we are able to control the transition rates along the different parts of the mRNA then we can steer the system to any desired ribosomal density profile. Also, controlling a single transition rate allows to steer the protein production rate to any desired value within a feasible range that is determined by the other, fixed transition rates. In both cases, using *fixed* rates is practically enough, and we show how these can be determined explicitly. These results are not trivial, as the occupation levels at different sites are interdependent due to the nonlinear excluded volume interactions between ribosomes.

Our results are based on the RFM that, as any mathematical model, is a simplification of (the biological) reality. For example, the RFM does not encapsulate some of the complex interactions between the transcript features and translation (see, e.g., [51], [42], [52]). Nevertheless, using the RFM allows one to pose the

controllability problem in a well-structured way, and study it rigorously using tools from systems and control theory. We believe that our analytical results may lead to new biological insights and suggest novel and interesting biological experiments. For example, it has been suggested that higher ribosome density contributes to a higher mRNA half life in *S. cerevisiae* [13]. However, it is difficult to determine if the correlation is due to a larger abundance of ribosomes along the entire coding region or maybe only the ribosome density at the 5' end of the coding region is relevant. It is also possible that this relation is due to a higher number of pre-initiation complexes at the 5'UTR (that contribute to a higher initiation rate). Specifically, it is possible that only higher pre-initiation density or ribosome density at the 5' end is important since the degradation starts from this region. Both factors are expected to correlate with higher ribosome density along the entire coding region, and a natural question is how can we design an experiment that can separate between the two possible explanations?

The results reported here suggest that we can design a synthetic library (that can be studied in-vitro and/or in-vivo) with different strains that have different initiation rates, but identical ribosome densities along the coding regions, or strains with different levels of ribosome densities at the first codons (or any other segment) of the coding regions, but similar ribosome densities in the rest of the coding region. Using such libraries may help in understanding exactly which factor contributes to the higher mRNA half life.

We believe that the results reported in this study may contribute in the future towards a better understanding of the molecular evolution of translation. Since usually a change in a transition rate is related to mutation/change in the mRNA codons composition, obtaining a desired ribosomal density profile and production rate involves introducing changes in the nucleotide composition of the transcript. Thus, an important future study should combine controllability analysis with models of molecular evolution.

Other topics for further research include the following. First, from the biological point of view a relevant problem is controllability when only some of the transition rates can be controlled, and each rate can take values in a discrete set of possible values only. Indeed, the admissible rates are limited by factors such as the concentrations of initiation and elongation factors, and the biophysical properties of the ribosome, mRNA, and translation factors. In this case, it is clear that we cannot obtain any desired density profile, and an interesting problem may be to determine the rate values that yield the “best” approximation for a given desired profile. This requires a biologically relevant definition of this best approximation, i.e., a measure of distance between two profiles that is biologically relevant.

Second, as noted above, the RFM is the mean-field approximation of TASEP. Our results naturally raise

the question of whether TASEP is controllable (in some stochastic sense). It is also interesting to examine if the analytical results obtained for the RFM can be used to synthesize suitable hopping rates for the stochastic TASEP model. In other words, suppose that we are given a desired profile P for the RFM, and determine the corresponding constant rates v_i s using (10). Does using these rates (perhaps after some normalization) as the TASEP hopping rates yield the steady-state profile P in TASEP as well?

Finally, TASEP has been used to model and analyze many other applications, for example, traffic flow. The RFM can also be used to study these applications, and controllability may be important here as well. For example, a natural question is can the density along a traffic lane be steered to any arbitrary profile by regulating speed signs along different sections of the lane?

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APPENDIX A: REVIEW OF CONTROLLABILITY

Controllability is a fundamental property of control systems, but it is not necessarily well-known outside of the systems and control community. For the sake of completeness, we briefly review this topic here. For more details, see e.g. [48].

Consider the control system

$$\begin{aligned}\dot{x} &= f(x, u), \\ y &= h(x, u),\end{aligned}\tag{18}$$

where $x : \mathbb{R}_+ \rightarrow \mathbb{R}^n$ is the state vector, $u : \mathbb{R}_+ \rightarrow \mathbb{R}^m$ is the control, and $y : \mathbb{R}_+ \rightarrow \mathbb{R}^k$ is the output. Let \mathbb{U} denote the set of admissible controls. Assume that the trajectories of this system evolve on a state space $\Omega \subseteq \mathbb{R}^n$. Given an initial condition $a \in \Omega$ and a desired final condition $b \in \Omega$, a natural control problem is: find a time $T \geq 0$, and an admissible control $u : [0, T] \rightarrow \mathbb{R}^m$ such that

$$x(T, u, a) = b.$$

In other words, u steers the system from a to b in time T . Of course, such a control may not always exist. This leads to the following definition.

Definition 1 System (18) is said to be *state-controllable* on Ω if for any $a, b \in \Omega$ there exist a time $T \geq 0$, and a control $u \in \mathbb{U}$ such that $x(T, u, a) = b$.

Sometimes it is enough to steer the output to a desired condition. This leads to the following definition.

Definition 2 System (18) is said to be *output-controllable* on some set $\Psi \subseteq \mathbb{R}^k$ if for any $p, q \in \Psi$ there exist a time $T \geq 0$, and a control $u \in \mathbb{U}$ that steers the output from $y(0) = p$ to $y(T) = q$.

Controllability is thus a theoretical property, but it is important in many applications, as it implies that the problem of determining a suitable control, i.e. the *control synthesis problem*, always admits a solution. From here on we focus on state-controllability. The notions for output-controllability are analogous.

Another useful notion, that is weaker than controllability, is called asymptotic controllability.

Definition 3 System (18) is said to be *asymptotically state-controllable* on Ω if for any $a, b \in \Omega$ there exists a control $u \in \mathbb{U}$ such that

$$\lim_{t \rightarrow \infty} x(t, u, a) = b.$$

Note that this implies that for any neighborhood V of b , there exists a time $T_s \geq 0$, and a control $u_s \in \mathbb{U}$ such that $x(T_s, u_s, a) \in V$.

For nonlinear control systems, analyzing controllability or asymptotic controllability is not trivial. There exists a weaker theoretical notion that can be analyzed effectively using Lie-algebraic techniques. For $a \in \Omega$, define the *reachable set from a* by

$$RS(a) := \{x(t, u, a) : t \geq 0, u \in \mathbb{U}\}.$$

In other words, $RS(a)$ is the set of all states that can be reached at some time $t \geq 0$ starting from $x(0) = a$. The system (18) is said to be *accessible* from a if the set $RS(a)$ has a non empty interior. In other words, the control is powerful enough to allow steering the trajectories emanating from a to a “full set” of directions.

Example 5 Consider the scalar system $\dot{x} = u$, with $\Omega = \mathbb{R}$. Let \mathbb{U} be the set of measurable functions taking non negative values for all time t . Pick $a \in \Omega$. Then $RS(a) = [a, \infty)$, so the system is accessible from a . However, the system is not controllable on Ω , as there does not exist any control $u \in \mathbb{U}$ that steers a to a point b with $b < a$. \square

Our results for the controlled RFM are based on showing that it is asymptotically state-controllable, using *constant* controls, and combining this with a Lie-algebraic sufficient condition for accessibility to deduce state-controllability.

To describe a sufficient condition for accessibility, consider the *control affine system*:

$$\dot{x} = f(x) + \sum_{i=1}^m g_i(x)u_i, \quad (19)$$

and assume that $0 \in \mathbb{U}$. For two vector fields $f, g : \mathbb{R}^n \rightarrow \mathbb{R}^n$, let $[f, g] := \frac{\partial g}{\partial x}f - \frac{\partial f}{\partial x}g$. This is another vector field called the *Lie-bracket of f and g*. For example, if $f(x) = Ax$ and $g(x) = Bx$ then $[f, g](x) = (BA - AB)x$. It is useful to introduce a notation for iterated Lie brackets. These can be defined inductively by letting $\text{ad}_f^0 g := g$, $\text{ad}_f^1 g := [f, g]$, and $\text{ad}_f^k g := [f, \text{ad}_f^{k-1} g]$ for any integer $k \geq 1$.

The *Lie algebra* A_{LA} associated with (19) is the linear subspace that is generated by $\{f, g_1, \dots, g_m\}$ and is closed under the Lie bracket operation. Let

$$A_{LA}(x_0) := \{p(x_0) : p \in A_{LA}\}.$$

Roughly speaking, it can be shown that if small-time solutions of (19) emanating from a point x_0 and corresponding to piecewise constant controls “cover” a k -dimensional set, with $k \leq n$, then $A_{LA}(x_0) = \mathbb{R}^k$. This yields the following sufficient condition for accessibility.

Theorem 2 *If $A_{LA}(x_0) = \mathbb{R}^n$ at some point x_0 then (19) is accessible from x_0 .*

The next result applies Theorem 2 to analyze accessibility in the RFM when either the entry rate or exit rate is replaced by a control.

Fact 1 *Consider the n -dimensional RFM with a single rate λ_i replaced by a scalar control $u(t)$. If $i = 0$ or $i = n$ then the control system is accessible from any point $x \in \text{int}(C^n)$.*

Proof of Fact 1. Consider the controlled RFM obtained by replacing λ_0 by $u(t)$, leaving the other rates as strictly positive constants. Let $z_0(x) := \lambda_0(1 - x_1)$, $z_j(x) := \lambda_j x_j(1 - x_{j+1})$, for $j = 1, \dots, n - 1$, and $z_n(x) := \lambda_n x_n$. The controlled RFM satisfies:

$$\dot{x} = f(x) + g(x)u, \quad (20)$$

where $f := \begin{bmatrix} -z_1 & z_1 - z_2 & z_2 - z_3 & \dots & z_{n-1} - z_n \end{bmatrix}'$, and $g := \begin{bmatrix} 1 - x_1 & 0 & \dots & 0 \end{bmatrix}'$. Let $p^k(x) :=$

$(\text{ad}_f^k g)(x)$. A calculation shows that for all $k \in \{0, \dots, n-1\}$,

$$p^k = \begin{bmatrix} p_1^k & \dots & p_k^k & p_{k+1}^k & 0 & \dots & 0 \end{bmatrix}',$$

with $p_{k+1}^k = (-1)^k \prod_{j=1}^{k+1} (1 - x_j) \prod_{\ell=1}^k \lambda_\ell$. Note that $p_{k+1}^k \neq 0$ for all $x \in \text{int}(C^n)$, so the n vector fields p^0, \dots, p^{n-1} are linearly independent, and thus span \mathbb{R}^n . Thus, the controlled RFM is accessible from any $x \in \text{int}(C^n)$.

Now consider the case where λ_n is replaced by a control $u(t)$. For $j = 1, \dots, n$, let $q_j(t) := 1 - x_{n+1-j}(t)$. Then

$$\begin{aligned} \dot{q}_1 &= (1 - q_1)u - \lambda_{n-1}q_1(1 - q_2), \\ \dot{q}_2 &= \lambda_{n-1}q_1(1 - q_2) - \lambda_{n-2}q_2(1 - q_3), \\ &\vdots \\ \dot{q}_n &= \lambda_1q_{n-1}(1 - q_n) - \lambda_0q_n. \end{aligned}$$

This is a controlled RFM with the initiation rate replaced by a control $u(t)$. It follows from the analysis above that this control system is accessible in $\text{int}(C^n)$, and this completes the proof. ■

Another sufficient condition for accessibility is based on *linearizing* the control system around an equilibrium point. For our purposes, it is enough to state this condition for the control affine system (19) with $m = 1$, i.e. the system:

$$\dot{x} = f(x) + g(x)u. \tag{21}$$

Theorem 3 [48, Ch. 3] *Suppose that $f(e) = 0$ and that $0 \in \text{int } \mathbb{U}$. Consider the linear control system*

$$\dot{z} = Az + ub,$$

where $A := \frac{\partial f}{\partial x}(e)$ and $b := g(e)$. If the $n \times n$ matrix $\begin{bmatrix} b & Ab & \dots & A^{n-1}b \end{bmatrix}$ has rank n then (21) is accessible from some neighborhood of e .²

²In fact, the condition above guarantees a stronger property, called first-order local controllability, but for our purposes the more restricted statement in Theorem 3 is enough.

Example 6 Consider the RFM with $n = 2$, i.e.

$$\dot{x}_1 = \lambda_0(1 - x_1) - \lambda_1 x_1(1 - x_2), \quad (22)$$

$$\dot{x}_2 = \lambda_1 x_1(1 - x_2) - \lambda_2 x_2,$$

with $\lambda_i > 0$. The equilibrium point e of this system satisfies $\lambda_0(1 - e_1) = \lambda_1 e_1(1 - e_2) = \lambda_2 e_2$. Suppose now that we can control the transition rate from site 1 to site 2. To study state-controllability in the neighborhood of e , consider the control system

$$\dot{x}_1 = \lambda_0(1 - x_1) - (\lambda_1 + u)x_1(1 - x_2), \quad (23)$$

$$\dot{x}_2 = (\lambda_1 + u)x_1(1 - x_2) - \lambda_2 x_2,$$

where \mathbb{U} is the set of measurable functions taking values in $[-\varepsilon, \varepsilon]$ for some sufficiently small $\varepsilon > 0$. This system is in the form (21) with $f(x) = \begin{bmatrix} \lambda_0(1 - x_1) - \lambda_1 x_1(1 - x_2) & \lambda_1 x_1(1 - x_2) - \lambda_2 x_2 \end{bmatrix}'$, and $g(x) = x_1(1 - x_2) \begin{bmatrix} -1 & 1 \end{bmatrix}'$. Note that $f(e) = 0$. To apply Theorem 3, calculate $A = \begin{bmatrix} -\lambda_0 - \lambda_1(1 - e_2) & \lambda_1 e_1 \\ \lambda_1(1 - e_2) & -\lambda_1 e_1 - \lambda_2 \end{bmatrix}$, $b = e_1(1 - e_2) \begin{bmatrix} -1 & 1 \end{bmatrix}'$, and

$$\begin{bmatrix} b & Ab \end{bmatrix} = e_1(1 - e_2) \begin{bmatrix} -1 & \lambda_0 + \lambda_1(1 - e_2) + \lambda_1 e_1 \\ 1 & -\lambda_1(1 - e_2) - \lambda_1 e_1 - \lambda_2 \end{bmatrix}.$$

Note that $\det(\begin{bmatrix} b & Ab \end{bmatrix}) = e_1^2(1 - e_2)^2(\lambda_2 - \lambda_0)$. Since $e \in \text{int}(C^2)$, Theorem 3 implies that if $\lambda_0 \neq \lambda_2$ then (23) is accessible in a neighborhood of e .

Now consider (23) with $\lambda_0 = \lambda_2$. Then $z := x_1 + x_2$ satisfies

$$\dot{z} = \lambda_0(1 - z).$$

Thus, any trajectory with $x_1(0) + x_2(0) = 1$ satisfies $x_1(t) + x_2(t) \equiv 1$ for any control u , and this implies that in this case (23) is not accessible and not state-controllable on C^2 . Summarizing, in this case the condition in Theorem 3 allows us to completely analyze the accessibility of (23). \square

This example may suggest that accessibility is lost when one of the *internal* (or elongation) rates λ_i , $i \in \{1, \dots, n - 1\}$, is replaced by a control, at least for some values of the other rates. However, the next example shows that is not necessarily true.

Example 7 Consider the RFM with $n = 3$, i.e.

$$\begin{aligned}\dot{x}_1 &= \lambda_0(1 - x_1) - \lambda_1 x_1(1 - x_2), \\ \dot{x}_2 &= \lambda_1 x_1(1 - x_2) - \lambda_2 x_2(1 - x_3), \\ \dot{x}_3 &= \lambda_2 x_2(1 - x_3) - \lambda_3 x_3,\end{aligned}$$

with $\lambda_i > 0$. Suppose that we can control the transition rate from site 1 to site 2, so we consider the control system:

$$\begin{aligned}\dot{x}_1 &= \lambda_0(1 - x_1) - x_1(1 - x_2)u, \\ \dot{x}_2 &= x_1(1 - x_2)u - \lambda_2 x_2(1 - x_3), \\ \dot{x}_3 &= \lambda_2 x_2(1 - x_3) - \lambda_3 x_3.\end{aligned}\tag{24}$$

We may ignore the term $x_1(1 - x_2)$ multiplying u , as it is strictly positive for all $x \in \text{int}(C^3)$. Thus, the control system is in the form (21) with $f(x) = \begin{bmatrix} \lambda_0(1 - x_1) & -\lambda_2 x_2(1 - x_3) & \lambda_2 x_2(1 - x_3) - \lambda_3 x_3 \end{bmatrix}'$, and $g(x) = \begin{bmatrix} -1 & 1 & 0 \end{bmatrix}'$. A calculation yields

$$v^1 := [f, g] = \begin{bmatrix} -\lambda_0 & \lambda_2(1 - x_3) & -\lambda_2(1 - x_3) \end{bmatrix}' ,$$

$$v^2 := [[f, [f, g]], [f, g]] = \begin{bmatrix} 0 & \lambda_2^2(\lambda_3(2 - x_3) - \lambda_2(1 - x_3)^2) & \lambda_2^2(\lambda_2(1 - x_3)^2 - \lambda_3) \end{bmatrix}' ,$$

and

$$v^3 := [v^2, v^1] = \begin{bmatrix} 0 & \lambda_2^3(\lambda_3 x_3 + \lambda_2(1 - x_3)^2) & -\lambda_2^3(\lambda_2(1 - x_3)^2 + \lambda_3) \end{bmatrix}' ,$$

so

$$\det \left(\begin{bmatrix} v^1 & v^2 & v^3 \end{bmatrix} \right) = 2\lambda_0 \lambda_2^5 \lambda_3^2 (1 - x_3).$$

Since this is different from zero for all $x \in \text{int}(C^3)$, we conclude that (24) is accessible from every $x \in \text{int}(C^3)$. \square

APPENDIX B: PROOFS

Proof of Theorem 1. We begin by defining a new control system obtained by replacing $\lambda_i, i \in \{0, \dots, n-$

1}], in the RFM (3) by a control function $u_i(t) : \mathbb{R}_+ \rightarrow \mathbb{R}_+$ (but leaving λ_n as a constant rate). This yields

$$\dot{x} = g_0(x) + \sum_{i=1}^n u_{i-1} g_i(x), \quad (25)$$

where $g_0(x) := [0 \ \dots \ 0 \ -\lambda_n x_n]'$, $g_1(x) := [1 - x_1 \ 0 \ \dots \ 0]'$, and for any $j \geq 2$, $g_j(x)$ contains the value $-x_{j-1}(1 - x_j)$ in its $(j - 1)$ 'th coordinate, the value $x_{j-1}(1 - x_j)$ in its j 'th coordinate, and the value 0 otherwise. For example, for $n = 4$:

$$\begin{aligned} g_0(x) &= [0 \ 0 \ 0 \ -\lambda_4 x_4]', \\ g_1(x) &= [1 - x_1 \ 0 \ 0 \ 0]', \\ g_2(x) &= [-x_1(1 - x_2) \ x_1(1 - x_2) \ 0 \ 0]', \\ g_3(x) &= [0 \ -x_2(1 - x_3) \ x_2(1 - x_3) \ 0]', \\ g_4(x) &= [0 \ 0 \ -x_3(1 - x_4) \ x_3(1 - x_4)]'. \end{aligned}$$

Pick $x^f \in \text{int}(C^n)$ and $z \in \mathbb{R}^n$. Then it is straightforward to show that

$$z = \sum_{i=1}^n \alpha_i g_i(x^f),$$

where

$$\alpha_i := \frac{\sum_{k=i}^n z_k}{x_{i-1}^f (1 - x_i^f)},$$

with $x_0^f := 1$. Note that since $x^f \in \text{int}(C^n)$, α_i is well-defined for all $i = 1, \dots, n$. We conclude that the vector fields $g_1(x^f), \dots, g_n(x^f)$ span \mathbb{R}^n . This implies, by known accessibility results (see, e.g. [48, Ch. 4]), that there exists a set $V = V(x^f) \subseteq \text{int}(C^n)$, that has a nonempty interior in \mathbb{R}^n , and such that every $p \in V$ can be steered to x^f in *finite time*. Fix arbitrary $q \in \text{int}(V)$ and $x^s \in C^n$. By Proposition 1, there exist constant controls u_0, \dots, u_{n-1} such that $\lim_{t \rightarrow \infty} x(t, u, x^s) = q$. Therefore there exists a time $\tau > 0$ such that $x(\tau, u, x^s) \in V$. The control $u(t)$, $t \in [0, \tau]$, can now be time-concatenated to a control w that steers $x(\tau, u, x^s)$ to x^f . Up to now, we only used $u = [u_0 \ \dots \ u_{n-1}]'$ (with $\lambda_n > 0$ fixed) and found a control u that steers $x(0) = x^s$ to $x(T) = x^f$. In particular, this control steers $x_n(0) = x_n^s$ to $x_n(T) = x_n^f$. Now replace λ_n by a control u_n , and set $u_n(t) \equiv \frac{R^f}{x_n^f}$. Then $R(T) = u_n(T)x_n(T) = R^f$, and this completes the proof. ■

Remark 3 Note that the construction above may lead to a production rate $R(t)$ that is discontinuous at $t = 0$. This can be easily overcome using any control $u_n(t)$ that smoothly interpolates between the values $u_n(0) := \frac{R^s}{x_n^s}$ and $u_n(T) := \frac{R^f}{x_n^f}$. For example, $u_n(t)$ could be picked linear in t . Also, we can take $u_n(t) \equiv \frac{R^s}{x_n^s}$ for $t \in [0, T - \varepsilon]$ and then a smooth interpolation between this value and $\frac{R^f}{x_n^f}$ on $t \in [T - \varepsilon, T]$. This way $u_i(t)$ is constant on $[0, T - \varepsilon]$ for all $i = 1, \dots, n$.

Proof of Proposition 2. Consider the RFM with rates $\lambda_0, \dots, \lambda_n$. It was shown in [33, Proposition 1] that R is a strictly increasing function of every λ_i . This means that in order to analyze Ω^i in the controlled RFM with $u \in \mathbb{U}$ it is enough to consider the reachable set for the controls $u(t) \equiv 0$ and $u(t) \equiv c$. It has been shown in [33] that for the rates $\lambda_0, \dots, \lambda_n$, the steady state production rate is $R = (\zeta_{MAX}(A(\lambda_0, \dots, \lambda_n)))^{-2}$. Thus for the two controls above $R(t)$ in the controlled RFM converges to 0 and to $M := (\zeta_{MAX}(A(\lambda_0, \dots, \lambda_{i-1}, c, \lambda_i, \dots, \lambda_n)))^{-2}$, respectively. We conclude that $\Omega^i = [0, M]$. ■

Proof of Proposition 3. Pick $R^f \in \text{int}(\Omega^i)$. Our goal is to show that there exist a finite time $T \geq 0$ and a control $u \in \mathbb{U}$ that steers $R(t)$ to R^f in time T . We consider two cases.

Case 1. Suppose that $i \neq n$. Since $R^f \in \text{int}(\Omega^i)$, there exists $\varepsilon > 0$ such that $(R^f - \varepsilon) \in \Omega^i$ and $(R^f + \varepsilon) \in \Omega^i$. Therefore, there exist $v^-, v^+ \in [0, c]$ such that for the control $u^-(t) \equiv v^-$ [$u^+(t) \equiv v^+$] the production rate converges to $R^f - \varepsilon$ [$R^f + \varepsilon$] for any x_0 . Applying u^- for a sufficiently long time T_1 yields $R(T_1) < R^f$. Now applying u^+ for a sufficiently long time T_2 yields $R(T_1 + T_2) > R^f$. Since $R(t)$ is continuous, this implies that there exists $T \in [T_1, T_1 + T_2]$ such that $R(T) = R^f$.

Case 2. Suppose that $i = n$, i.e. $R(t) = u(t)x_n(t)$. The argument used in Case 1 does not hold as is because now a discontinuity in u yields a discontinuity in $R(t)$. However, it is clear that we can design a control u by concatenating $u(t) \equiv v^-$ for $t \in [0, T_1]$, then a function of time satisfying $u(T_1) = v^-$ and $u(T_1 + \tau) = v^+$, with $\tau > 0$, and finally $u(t) \equiv v^+$ for $t \geq T_1 + \tau$, and that this will steer $R(t)$ to R^f at some final time T . ■

Proof of Proposition 4. It has been shown in [33] that $\frac{\partial R}{\partial \lambda_i}$ exists and is strictly positive for all $i \in \{0, \dots, n\}$. Combining this with (6) implies that $\frac{\partial e_k}{\partial \lambda_i}$ exists for all $k \in \{1, \dots, n\}$ and all $i \in \{0, \dots, n\}$.

Pick $i \in \{1, \dots, n-2\}$. Differentiating (6) with respect to λ_i yields

$$\begin{aligned}
-\lambda_0 e'_1 &= \lambda_1 e'_1 (1 - e_2) - \lambda_1 e_1 e'_2 \\
&= \lambda_2 e'_2 (1 - e_3) - \lambda_2 e_2 e'_3 \\
&\vdots \\
&= \lambda_{i-1} e'_{i-1} (1 - e_i) - \lambda_{i-1} e_{i-1} e'_i \\
&= e_i (1 - e_{i+1}) + \lambda_i e'_i (1 - e_{i+1}) - \lambda_i e_i e'_{i+1} \\
&= \lambda_{i+1} e'_{i+1} (1 - e_{i+2}) - \lambda_{i+1} e_{i+1} e'_{i+2} \\
&\vdots \\
&= \lambda_{n-1} e'_{n-1} (1 - e_n) - \lambda_{n-1} e_{n-1} e'_n \\
&= \lambda_n e'_n \\
&= R',
\end{aligned} \tag{26}$$

where we use the notation $f' := \frac{\partial f}{\partial \lambda_i}$. Since $R' > 0$, we conclude that $e'_1 < 0$. Now the equation $\lambda_1 e'_1 (1 - e_2) - \lambda_1 e_1 e'_2 = R'$, and the fact that $e \in (0, 1)^n$ yield $e'_2 < 0$. Continuing in this fashion yields $e'_j < 0$ for all $j \leq i$. The last equality in (26) yields $\lambda_n e'_n > 0$, so $e'_n > 0$. Now the equality $\lambda_{n-1} e'_{n-1} (1 - e_n) - \lambda_{n-1} e_{n-1} e'_n = R'$ yields $e'_{n-1} > 0$, and continuing in this fashion yields $e'_j > 0$ for all $j > i$. This completes the proof for the case $i \in \{1, \dots, n-2\}$. The proof when $i \in \{0, n-1, n\}$ is similar. ■

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