Mediating Ribosomal Competition by Splitting Pools

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Abstract—Synthetic biology constructs often rely upon the introduction of “circuit” genes into host cells, in order to express novel proteins and thus endow the host with a desired behavior. The expression of these new genes “consumes” existing resources in the cell, such as ATP, RNA polymerase, amino acids, and ribosomes. Ribosomal competition among strands of mRNA may be described by a system of nonlinear ODEs called the Ribosomal Flow Model (RFM). The competition for resources between host and circuit genes can be ameliorated by splitting the ribosome pool by use of orthogonal ribosomes, where the circuit genes are exclusively translated by mutated ribosomes. In this work, the RFM system is extended to include orthogonal ribosome competition. This Orthogonal Ribosomal Flow Model (ORFM) is proven to be stable through the use of Robust Lyapunov Functions. The optimization problem of maximizing the weighted protein translation rate by adjusting allocation of ribosomal species is formulated.

I. INTRODUCTION

The process of protein expression is mediated by ribosomes. After a gene in DNA has been transcribed into messenger RNA (mRNA), a ribosome binds to the mRNA and begins protein translation. The mRNA is divided into a set of 3-nucleotide segments called codons, and each codon corresponds to an amino acid or a stop instruction. The ribosome attracts a tRNA carrying an amino acid which matches the currently read codon, and appends it to a growing polypeptide chain. Once the ribosome hits a stop codon, it falls off the mRNA and releases the amino acid chain as a polypeptide, which is subsequently post-translationally processed into a final protein product. The mRNAs remain in the cell until they are degraded or destroyed (such as by small interfering RNA). Intact mRNAs continually attract ribosomes and produce protein, and all mRNAs in the cell compete for a finite pool of resources which includes ribosomes. A substantial literature exists on the problem of gene expression burden including ribosome competition; see for example the many references reviewed in [1]. When new (circuit) genes are implanted into a cell, the circuit genes compete for ribosomes with the original (host) genes. One way to decouple host and circuit gene expression is to split the pool of resources via orthogonal ribosomes [2], [3], [4]. Orthogonal ribosomes are mutated ribosomes which only translate specifically modified circuit genes. One such construction replaces the 16S-rRNA (component of small ribosomal subunit) in E. coli with a synthetic version. The circuit’s mRNAs binding sites are designed so that only mutated ribosomes will translate circuit mRNAs; host ribosomes are not attracted to the circuit mRNAs [5]. Orthogonal ribosomes may be able to decrease competition and increase protein throughput by splitting the pool.

We present the Orthogonal Ribosomal Flow Model (ORFM) extending existing RFM network models with ribosome competition. Global asymptotic stability of the ORFM is certified by means of Robust Lyapunov Functions. A bisection algorithm is detailed to compute the unique ORFM steady state. The protein throughput of the system can be changed by adjusting the production rate of ribosomal species.

The structure of the paper is as follows: Section II reviews existing models for protein translation and ribosome competition. Section III introduces the ORFM. Section IV presents an optimization problem to maximize protein throughput. Section V adds a feedback controller to regulate production of ribosomes. Section VI concludes the paper. The stability of the ORFM is proved in the Appendix.

II. RIBOSOMAL FLOW MODELS (RFMs)

A. Single-Strand Translation Models

The RFM [6] is a deterministic mean-field approximation to lattice models of steady-state ribosome distribution on mRNAs [7], [8]. Each codon on the length-n mRNA has a normalized ribosomal density $x_j(t) \in [0, 1]$ for $j = 1, \ldots, n$, which one may think of as the probability that a ribosome is present on codon $j$ at time $t$. Transition rates between codons are denoted here as $\lambda_j$. The initiation rate $\lambda_0$ is the rate at which the mRNA attracts the ribosome to begin translation, while the $\lambda_{j+1}$ are elongation rates and represent the amount of time required for the ribosome to attract the codon’s respective tRNA. Finally, $\lambda_n$ is the rate at which ribosomes separate from the mRNA and release the completed polypeptide chain. The quantity $y = \lambda_n x_n$ is the rate (protein/time) at which product is produced by the mRNA.

An RFMIO (input/output) is a tridiagonal polynomial single-input single-output system

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

$$
\dot{x}_j = \lambda_{j-1} x_{j-1} (1 - x_j) - \lambda_j x_j (1 - x_{j+1}), 1 < j < n
$$

$$
\dot{x}_n = \lambda_{n-1} x_{n-1} (1 - x_n) - \lambda_n x_n
$$

$$
y = \lambda_n x_n.
$$

The output $y$ is the translation rate, and the input $u$ is the rate at which new ribosomes become available. The probability that codon $j$ is empty is $x_j$, and the probability that codon $j+1$ is empty is $1 - x_{j+1}$. The likelihood that ribosomes flow from codons $j$ to $j+1$ is proportional to $x_j (1 - x_{j+1})$, which is the density relation times the elongation rate in (1).
For a constant input \( u > 0 \), there is a unique steady-state in \([0, 1]^n\) for system (1), which we denote as \( e \). Figure 1 shows the steady state of an 5-codon RFM. Each codon is a black box, and \( x_j \) is the filled proportion of each codon. Ribosomes flow from left to right, and the bars between codons show the flux rates \( \lambda_j x_j (1 - x_{j+1}) \) (which must be all equal, as is clear from the equations). The steady state codon occupancies \( e_j \) and output \( y = \lambda_n e_n \) may be computed by solving a finite continued fraction, which results in a polynomial equation of degree \((n + 1)/2\) [6]. A spectral formulation for the constant \( u \) was presented by Poker et. al., and is reviewed in Algorithm 1. The operator \( \text{tridiag}(\alpha, \beta) \) for \( \alpha, \beta \in \mathbb{R}^{n-1} \) produces a symmetric \( b \times b \) tridiagonal matrix with main diagonal \( \alpha \) and 1-off-diagonals \( \beta \). For a square symmetric matrix \( M \), \( \sigma_{\text{max}}(M) \) and \( v_{\text{max}}(M) \) are its largest eigenvalue and respective eigenvectors. The vector \( \zeta \) is the Perron-Frobenius eigenvector of the Jacobi matrix formed by the rates \( \lambda \) (dominant eigenvector with nonnegative entries).

**Algorithm 1: RFMIO Steady State (from [9])**

```plaintext
input : Rates \( \lambda \), Constant Input \( u \\
output: \text{Codon Steady States } e_j \\
\mu_j = 1/\sqrt{\lambda_j} \text{ for } j = 0, \ldots, n \\
J = \text{tridiag}(0, \mu) \\
\sigma = \sigma_{\text{max}}(J), \ \zeta = v_{\text{max}}(J) \\
e_j = \frac{\mu_j \zeta_{j+1}}{\sigma} \text{ for } j = 1, \ldots, n
```

The RFM system is a monotone control system. The set of admissible controls \( u \in \mathcal{U} \) is the set of bounded and measurable functions \( u \in \mathbb{R}_+ \rightarrow \mathbb{R}_+ \). The RFM is state and output-controllable, and desired translation rates and patterns can be achieved by proper choice of \( u \) and \( \lambda \) [10].

**B. Ribosomal Competition**

In real genetic systems, all mRNA’s in the cell compete for a finite (and possibly time-varying) number of ribosomes. Particularly slow transition rates \( \lambda \) on codons can lead to strands of mRNA hoarding ribosomes, reducing the availability of ribosomes in the pool for other mRNA’s and leading to a globally depressed translation rate.

Raveh et. al. introduced a ribosomal flow model network with a pool (RFMNP) to abstractly describe the impact of ribosomal competition with multiple strands of mRNA [11]. Each of the \( s \) strands of mRNA is modeled by a RFMIO \( (x_j^i) \) for codon \( j \) of mRNA \( i \), and all RFMOI are connected to a common pool \( z \). The total number of ribosomes in the system \( N = z(t) + \sum_{i,j} x_j^i(t) \) is conserved. The input \( u \) of each mRNA is an increasing saturation function \( G^i(z) \) (commonly \( z \) or \( \tanh(z) \)), which describes the likelihood that a ribosome from the pool will attach itself to strand \( i \). The translation rate \( y_j^i = \lambda_n^i x_j^i \) is the output, and ribosomes leaving \( x_j \) return to the pool. The pool dynamics are therefore

\[
\dot{z} = \sum_i \lambda_n^i x_n^i - \sum_i \lambda_n^i G^i(z)(1 - x_j^i) .
\]

(2)

The RFMNP is a closed loop system. The number of ribosomes \( N \) defines a stoichiometric class, and RFMNP has a globally asymptotic equilibrium point with all \( e_j^i \in (0, 1) \) and \( z \in (0, N) \) for each \( N \). Stability can be proven by contraction, where the weighted \( L_1 \) norm between trajectories is non-expanding over time [11].

**III. ORTHOGONAL RIBOSOMAL FLOW MODEL**

Orthogonal ribosomes can be added to the RFM scheme (ORFM) by splitting the ribosome pool \( z \). Code for ORFM simulation and visualization is publicly available online at [https://gitlab.com/jarmill/Ribosomes](https://gitlab.com/jarmill/Ribosomes).

**A. ORFM Formulation**

The proposed model of ribosome translation has \( M \) species of ribosomes. Each ribosome species has a pool \( z_p \) for \( p = 1, \ldots, M \) of available ribosomes. Strands of mRNA that use ribosome type \( p \) form an RFMNP with pool \( z_p \).

Ribosomes of type \( p \) are formed by the combination of a 16S-rRNA of type \( p \) and the remaining ribosome components. The protein backbone and large ribosomal subunit are treated as an ‘Empty’ ribosome \( E \), which has no translation capacity on its own. Empty ribosomes bind with rRNA at the rate...
Algorithm 2: ORFM Steady State

input : \( \lambda_{pi}, N, G_{pi}, K_p, \epsilon \)
output: Steady States \( z_E, z_p, e_{ji} \)
\( \bar{N}_{min} = 0, \bar{N}_{max} = N \)
repeat
| \( \bar{N}_{mid} = (\bar{N}_{max} + \bar{N}_{min})/2 \) |
| \( z_E = \frac{\bar{N}_{mid}}{\sum_p \bar{x}_{pi}} \) |
| \( z_p = \frac{\bar{K}_p \bar{N}_{mid}}{\sum_p \lambda_{pi}} \) |
| \( e_{ji} = \text{RFMIO SS}(\lambda_{pi}, G_{pi}(z_p)) \) (Alg. 1 \( \forall p, i \)) |
| \( N_{curr} = \bar{N}_{mid} + \sum_{p,i,j} e_{ji} \) |
| if \( N_{curr} \leq N \) then |
| | \( \bar{N}_{min} \rightarrow \bar{N}_{mid} \) |
| else |
| | \( \bar{N}_{max} \rightarrow \bar{N}_{mid} \) |
until \( |N_{curr} - N| \leq \epsilon \);

\( k_p^+ \), and the ribosomal complex dissociates at the rate \( k_p^- \), assuming rRNA’s abundance. These kinetics are inspired by Darlington et al.‘s mass-action model of protein translation and cell metabolism [12]. The \( M \) pools of translating ribosomes \( z_p \) are each connected to the pool of empty ribosomes \( z_E \). Each of the \( s_p \) strands of mRNA that obtain ribosomes from pool \( z_p \) will have a corresponding length \( n_p \). Copies of mRNA with identical initial conditions are represented as a single strand with multiplicity \( m_p \). The codon at location \( j \in 0, \ldots, n_p - 1 \) of RNA strand \( i \in 1, \ldots, s_p \) coming from pool \( p \) is \( z_{pi} \). The constants of this system are the \( N \) ribosomes, binding rates \( k_p^+ \) and \( k_p^- \), and transition rates \( \lambda_{pi}^p \).

The total number of ribosomes \( N \) is a conserved quantity
\[
N = z_E + \sum_{p=1}^{M} z_p + \sum_{p=1}^{M} \sum_{i=1}^{s_p} \left( m_p n_p \sum_{j=1}^{n_p} z_{pi} \right) = \sum_{p=1}^{M} \sum_{i=1}^{s_p} n_p z_{pi}. \tag{3}
\]

The translation dynamics are
\[
\dot{z}_E = \sum_{p=1}^{M} k_p^+ z_p - \sum_{p=1}^{M} k_p^- z_E \tag{4a}
\]
\[
\dot{z}_p = k_p^+ z_E - k_p^- z_p + \sum_{i=1}^{s_p} m_p n_p \lambda_{pi}^p n_p \sum_{j=1}^{n_p} z_{pi} \tag{4b}
\]
\[
\dot{x}_{pi}^p = \lambda_{pi}^p (1 - x_{pi}^p) G_{pi}(z_p) - \lambda_{pi}^p (1 - x_{pi}^p) x_{pi}^p \tag{4c}
\]
\[
\dot{x}_{pi}^p = \lambda_{pi}^p (1 - x_{pi}^p) x_{pi}^p - \lambda_{pi}^p (1 - x_{pi}^p) x_{pi}^p \tag{4d}
\]
\[
\dot{x}_{pi}^p = \lambda_{pi}^p (1 - x_{pi}^p) x_{pi}^p \tag{4e}
\]

Let \( \bar{N} = z_E + \sum_{p=1}^{M} z_p \) be the number of the pool ribosomes at steady state. For a fixed \( \bar{N} \), the steady state pool occupancies \( z_p, z_E \) can be obtained by solving the linear system
\[
0 = \dot{z}_E + \sum_{p=1}^{M} m_p n_p \dot{x}_{pj}^p, \quad p = 1, \ldots, N_p \quad \text{where} \quad K_p = k_p^+ / k_p^-
\]
\[
z_p = K_p z_E, \quad \bar{N} = z_E + \sum_{p=1}^{M} z_p. \tag{5}
\]

Algorithm 2 computes an approximation to the steady-state of the general model by bisection on \( \bar{N} \). It converges faster than numerical integration, which is a significant difference in the optimization routine. A built-in MATLAB solver (fzero) may find a point that is outside the valid region. Fig. 3 shows examples of mRNA competing for \( N = 10 \) ribosomes, where each mRNA may take ribosomes from either the host pool \( (p = 1, \text{blue}) \) or the circuit pool \( (p = 2, \text{red}) \). The ‘empty’ ribosomes are displayed in purple in the third pool. In this network, \( k_p^+ = k_p^- = 1 \), so at steady state, the pool quantities \( z_1 = z_2 = z_E \). In Figure 3a no mRNA takes ribosomes from the circuit pool \( z_2 \), so the ribosomes in \( z_2 \) induce deadweight loss for the system.

Fig. 4 illustrates a general model orthogonal ribosomal system with pool across three ribosomal subspecies and \( N = 20 \). Now \( k_p^+ = k_p^- = 0.1 \), \( p = 1, 2, 3 \), so all pools will have an equal number of ribosomes at equilibrium \( (z=0.286) \).

B. Stability of the ORFM

The RFM has recently been studied by constructing explicit Robust Lyapunov Functions (RLFs) [13] via writing it as a Chemical Reaction Network (CRN) and utilizing relevant methods [14], [15]. Such techniques provide explicit formulæ of Lyapunov functions for general kinetics (not limited to Mass-Action), and have an easy-to-use software package. In this subsection, we derive the stability of the ORFM model (4a)-(4e) via an RLF. Let \( x := [x_{pi}]_{i,j,p} \in [0, 1]^N \) be the vector of all codon occupancies, and \( \bar{N}_c \) be the total number of codons. Let \( z := [z_1, \ldots, z_M, z_E] \in [0, N]^{M+1} \) be the vector of all pool occupancies. We therefore have:

\textit{Theorem 1:} Consider the system (4a)-(4e). Then,
(1) The function:
\[
V(x, z) = \sum_{p,i} m_p n_p \sum_{j} x_{pi}^p + \sum_{p} |z_p| + |z_E|,
\]
is a (non-strict) Lyapunov function for any choice of \( \lambda_{pi} \) > 0 and monotone functions \( G_{pi} \), and
(2) For any fixed total number of ribosomes in the system \( N_c > 0 \), there exists a unique positive globally asymptotically stable steady-state \( (x_c, z_c) \) for (4a)-(4e).

A proof of Theorem 1 is given in the Appendix. Alternatively, the system (4a)-(4e) can be transformed into a CRN,
and Theorem 1 can be verified via the software package LEARN [16] for a fixed number of pools, strands, and codons.

IV. OPTIMIZATION

This section poses an optimization problem to maximize the weighted sum of protein production by mRNA. Optimization of protein throughput in a single strand of mRNA has previously been treated by Pokry et al. in [9]. Given an n-codon mRNA with allowable choices of \( \lambda_i \) in a convex set, finding rates \( \lambda \) that maximize the throughput \( y = \lambda_n c_n \) is a concave optimization problem. This section adjusts rates \( K \) with constant \( \lambda \) to maximize a weighted-sum objective is \( y_w = \sum_{p,i} w^p_i m^p_i p^p \). Appropriate selection of the weights \( w \) can specify particular proteins as desirable. As a problem setting, consider an ORFM with \( M \) species and \( N \) ribosomes. There will be \( M + 1 \) pools: \( z_E \) for the empty ribosomes and \( z_p \) for the translating ribosomes. If there exists sufficient flexibility to adjust \( K \), then \( K_p \in [K_p^\text{min}, K_p^\text{max}] > 0 \), then \( K_p \in [K_p^\text{min}, K_p^\text{max}] = \left[ \frac{k_p^{\text{min}}}{k_p^{\text{max}}}, \frac{k_p^{\text{max}}}{k_p^{\text{min}}} \right] \). It is assumed that \( 0 < K_p^\text{min} \leq K_p^\text{max} < \infty \) for each species \( p \). For each point \( K = \{K_p\}_{p=1}^{M} \) the weighted protein output \( y_w(K) \) can be obtained by finding the ORFM steady state with respect to \( K \) using Algorithm 2 and then evaluating \( y_w = \sum_i w^p_i m^p_i p^p \). An optimization problem to maximize \( y_w \) at steady state can therefore be formulated:

\[
y_w^* = \max_K \sum_{p,i} w^p_i m^p_i p^p, \tag{6}
\]

subject to : \( K_p \in [K_p^\text{min}, K_p^\text{max}] \quad \forall p = 1, \ldots, M \)

Steady state of dynamics in (4a)-(4e).

The search variables of problem (6) are \( K_p \), and the steady states \( (z_E, z_p, c_p) \) are derived quantities of \( K_p \). Fig 5 shows an ORFM with \( s_1 = 6 \) and \( s_2 = 3 \) strands with an objective of maximizing total protein output \( (w = 1) \). If all mRNA were connected to a common pool of ribosomes (RFMNP), the resultant output is \( y_w = 3.18 \). When \( K = [5,5] \), then \( y_w = 3.11 \) as shown in Fig. 5a. Solving problem (6) with a grid search for \( K^{\text{min}} = \frac{1}{5} \) and \( K^{\text{max}} = 5 \) results in the optimum rates \( K^* = [5,0.734] \) and protein output \( y_w^* = 3.38 \) in Fig. 5b. The optimization landscape of \( y_w \) vs. \((\log_{10}(K_1), \log_{10}(K_2))\) is displayed in Fig. 6.

A. Parameter Effects

This subsection analyzes the effects on properties of the ORFM steady state by incrementally changing a rate \( K_p \). For a single strand \( x^p \) taking ribosomes from pool \( z_p \), the effective intake rate from Eq. (1) is \( \lambda_0 G(z) \). Define the throughput \( y^p_i(z_p) \) as the protein output in strand \( p \) given pool occupancy \( z_p \), and \( N^p_i(z_p) = \sum_j c_{pj}^i \) as the ribosome occupancy of strand \( p \) given \( z_p \). Since \( G(z) \) is monotonically increasing and through results in [9], the functions \( y^p \) and \( N^p_i \) are both nonnegative increasing functions of \( z_p \). Let the weighted output \( y_w^p(z_p) = \sum_i w^p_i m^p_i p^p(z_p) \), and the number of ribosomes of species \( p \) be \( N_p(z_p) = z_p + \sum_i m^p_i N^p_i(z_p) \) across all strands of species \( p \). The total weighted output is \( y_w = \sum_p y_w^p(z_p) \), and the occupancy is \( N = z_E + \sum_p z_p + \sum_{p,i,j} m^p_i c_{pj}^i = z_E + \sum_p N_p(z_p) \).

As a shorthand let \( \partial = \partial_{K_p} \) for a chosen species \( p \), \( N_i' = dN_i'/dz \), and \( y_{wp}' = dy_{wp}/dz \). From Eqs. (5) and (7)

\[
0 = \partial(K_p z_E - z_p) = z_E + K_p \partial z_E - \partial z_p \tag{8a}
\]

\[
0 = \partial(K_p z_E - z_p) = K_p \partial z_E - \partial z_p', \quad \forall p' \neq p \tag{8b}
\]

\[
0 = \partial_n = \partial z_E + \sum_{p'=1}^M \partial N_p'(z_p) \tag{8c}
\]

By the chain rule, \( \partial N_p(z_p) = N_p'(z_p) \partial z_p \). The quantity \( \partial z_E \) can be found from Eqs. (8a), (8b), and (8c)

\[
0 = \partial z_E + \sum_{p'=1}^M \partial z_p N_p'(z_p) \tag{9a}
\]

\[
= \partial z_E + z_E N_p'(z_p) + \sum_{p'=1}^M K_p' N_p'(z_p) \partial z_E \tag{9b}
\]

\[
\partial z_E = -z_E N_p'(z_p) < 0. \tag{9c}
\]

The change \( \partial z_E < 0 \) because all values \( z_E, K_p, N_p'(z_p) > 0 \) over the valid range of \( K \). Likewise \( \partial z_p' = K_p \partial z_E < 0 \) for \( p \neq p' \). In contrast,

\[
\partial z_p = z_E \left( 1 - \frac{K_p N_p'(z_p)}{1 + \sum_{p'=1}^M K_p' N_p'(z_p')} \right) > 0. \tag{10}
\]

Plugging back into the chain rule, \( \partial N_p'(z_p) = N_p'(z_p) \partial z_p' > 0 \) and \( \partial N_p'(z_p') = N_p'(z_p') \partial z_p' < 0 \) for \( p' \neq p \). This is an intuitive conclusion: increasing the production rate of species \( p \) will increase the number of ribosomes of type \( p \) at the expense of all other species (including \( z_E \)).

The change in objective \( y_w \) by increasing \( K_p \) is

\[
\partial y_w = \partial z_E y_{wp}'(z_p') + \partial z_E \sum_{p'=1}^M K_p' y_{wp}'(z_p'). \tag{11}
\]

The \( z_E \) term of (11) is \( > 0 \) while the \( \partial z_E \) term is \( < 0 \). The sign of \( \partial y_w \) may change over the valid region of \( K \). Problem (6) is generically a non-concave problem in terms of \( K \), and
may feature more than one local maximum depending on the choice of $w$. Standard approaches of global optimization such as Grid Search, Bayesian Optimization, and Basin Hopping can be used to approximate $K^*$, where the cost function $y_w(K)$ at each $K$ is evaluated by Algorithm 2.

V. SELF-INHIBITING FEEDBACK CONTROLLER

The introduced optimization framework adjusts the ribosomal production rates $K$ to maximize the weighted protein output $y_w$ (6) assuming perfect knowledge of systems and parameters. However, if the transition rates and the number of mRNA strands per pool are subject to changes or are not known a priori, we propose a controller inspired by [12] to regulate the expression of ribosomes by self-inhibiting feedback. For instance, the system in Fig. 3b with high circuit demand can be optimized by (6) to maximize the total amount of protein ($w = 1$). If the circuit demand drops as shown in Fig. 3a, circuit ribosomes will be maintained without being used in translating protein (high $K_2$). Changing the system demand would require re-optimization of $K$.

Alternatively, a feedback controller can be introduced to dynamically adjust the previously constant $K$, only producing ribosomes of species $p$ when there exists corresponding demand. This can be accomplished by creating one distinguished mRNA with codon occupancies $x^{pF}_j$ per species $p$. The new mRNA is translated into a protein $F_p$. The dynamics of the protein $F_p$ with a degradation rate $\delta_p$ is

$$F_p = \lambda^{pF}_n x^{pF}_n - \delta_p F_p.$$  (12)

A Hill-like inhibition term can be used to suppress the creation of new ribosomes (based on [12, S1-(18)]). For a nominal ribosome creation rate $k^{p0}_n$, exponent $\gamma_p$, and constant $x^{p0}_j$, the suppressed $k^{p+}_n$ is: $k^{p+}_n = k^{p0}_n/(1 + F_p/F^{p0}_p)^{\gamma_p}$. In summary, $x_p$ activates $F_p$ while $F_p$ inhibits $x_p$. The controller is deemed to perform as desired if the number of circuit ribosomes adjusts according to the circuit demand. Removing the circuit from the system would initially raise $F_p$, which represses $k^{p+}_n$ strongly and hence reduces the number of circuit ribosomes. If circuit genes are introduced, the new genes will compete with the inhibitor mRNA and the number of circuit ribosomes will increase.

Fig. 7 shows an example of an ORFM with 10 ribosomes, where the circuit pool has a feedback controller. The inhibitor protein $F_2$ is translated from the golden two-codon mRNA with occupancies $x^{2F}_j$ and rates $\lambda^{2F} = [0.005, 5, 5]$. The low initiation rate $\lambda^{2F}_n = 0.005$ allows other mRNA if present to take ribosomes from $z_2$ first. The inhibitor parameters are $F_2 = 1.5$, $\gamma_2 = 4$, and $\delta_2 = 0.01$. The golden sheath in Fig. 7 between $z_2$ and $z_E$ represents the nominal rate $k^{p+}_n z_E$, compared to the purple core’s inhibited $k^{p+}_n z_E$.

With no circuit demand, the circuit ribosome creation rate in Fig 7a falls from 1.393 to 0.669 as desired.

Let $N_1 = z_1 + \sum_{i,j} x^{p1}_j$ be the number of host ribosomes (species 1), and $y_w$ be the total non-inhibitory protein output (excluding $F_2$). With no inhibitor or circuit mRNA, the system in Fig. 3a has an expected $N_1 = 7.63$ and $y_w = 3.89$. With the inhibitor in Fig. 7a, the expected total number of host ribosomes rises to $N_1 = 7.97$, $y_w = 3.96$. Fig. 3b has a high circuit demand and no inhibitor, and $N_1 = 3.94$, $y_w = 4.98$. With an inhibitor in Fig. 7b, $N_1 = 4.41$, $y_w = 5.06$. At no circuit demand the steady state $F_2 = 0.292$ units of inhibiting protein, and at high circuit demand $F_2 = 0.138$ units.

VI. CONCLUSION

Competition for finite resources are inevitable in protein translation. Orthogonal ribosomes have been developed to boost protein throughput by decoupling circuit genes from the host pool of ribosomes. We extended the existing RFM to orthogonal ribosomes, and generalized the system to an arbitrary number of ribosomal species. Stability results through RLFs and a simple algorithm to compute steady states were presented. Maximizing the weighted sum of protein throughput can be formulated as an optimization problem. A self inhibiting feedback controller can adjust ribosomal production as needed. Future work includes matching results with lab experiments and further development of the feedback controller. The problem of cross-talk in translation is discussed in an extended version of this paper [17].

APPENDIX: PROOF OF THEOREM 1

For simplicity, assume that $s_p = s, n^{p,i} = n$, for all $p, i$. Also, assume $m^{p,i} = 1$, for all $p, i$. The argument for the general case will be the similar. Denote $\sigma(x) := \text{sgn}(x)$.

To use the techniques in [15], [13], we lift (4a)-(4e) to a higher-dimensional space by defining the vacancy $w^{p,i}_{j-1} := 1 - x^{p,i}_{j-1}$, for all $j, i$. Hence, terms of the form $(1 - x^{p,i}_{j-1})w^{p,i}_{j-1}$ take the familiar Mass-Action form: $w^{p,i}_{j-1} x^{p,i}_{j}$. We will generalize this further by considering arbitrary monotone functions of the form $R(w^{p,i}_{j-1}, x^{p,i}_{j})$. Hence, we write (4a)-(4e) as:

$$\dot{z}_E = \sum_p R^{-}_p(z_p) - \sum_p R^{+}_p(z_E)$$

$$\dot{z}_p = R^{+}_p(z_E) - R^{-}_p(z_p) + \sum_i \left( R^{p,i}_n(x^{p,i}_n) - R^{0,p}_0(w^{p,i}_1, z_p) \right)$$

$$x^{p,i}_{j-1} = R^{p,i}_j(x^{p,i}_j, z_p) - R^{p,i}_{j-1}(w^{p,i}_{j-1}, x^{p,i}_{j-1})$$

$$x^{p,i}_n = R^{p,i}_{n-1}(w^{p,i}_{n}, x^{p,i}_{n-1}) - R^{p,i}_n(z_k)$$  (13)

We only assume that the rates $R^{p,i}_j, R^{p,i}_n$ are monotone w.r.t their reactants, see [13] for the full assumptions.

Consider $V(x, z) = \sum_{i,j} |x^{p,i}_j|^2 + \sum_{p,j} |z_p|^2 + |z_E|^2$. We show that $V$ is non-increasing. Using (13), note that the space can be partitioned into regions, and $V$ is linear in rates on each region. Fix an open region $W$ and write $V = \sum_{p,i,j} \alpha^{p,i}_j R^{p,i}_j(x, z) + \sum_p \beta_p(R^{p}_p(z_p) - R^{0}_p(z_E))$ on $W$.
Note \( V \) is differentiable on \( W \), and the signs of the currents \( \dot{x}_{pi}^j, \dot{z}_p, \dot{z}_E \) are constant on \( W \). We claim that the Jacobian \( Q \) for which the (reduced) Jacobian is non-degenerate to show the structure and are independent of the rates. Furthermore, it has been shown also [13] that the existence of an RLF implies that it sufficient to find one positive point \( p^* = (p_1^*, \ldots, p_n^*) \) for which the (reduced) Jacobian is non-degenerate to show that is non-degenerate for all \( \rho \geq 0 \). We will find that point next by studying the structure of the Jacobian.

Similar to a single pool [11, SI], it can be easily seen that \( J \) has non-negative off-diagonals and strictly negative diagonals (i.e. \( J \) is Metzler). In addition, conservation of the number of ribosomes implies that \( 1^T J = 0 \). By the definition of the Jacobian, all the entries in each column contain only the partial derivatives with respect to the state variable associated to the column. Hence, we can choose the corresponding \( p^*_i \)'s such the diagonal entry in each column is scaled to \(-1\). Therefore, we consider the Jacobian evaluated at the chosen point \( \rho^* \) such that \( J^* = P - I \), where \( I \) is the identity matrix and \( P \) a nonnegative irreducible column-stochastic matrix.

By Perron-Frobenius Theorem, \( P \) has a maximal eigenvalue \( 1 \) with algebraic multiplicity 1. Therefore, \( J^* \) has a single eigenvalue at 0 and the remaining eigenvalues have strictly negative real-parts. Hence, the reduced Jacobian at \( \rho^* \) is non-degenerate. Robust non-degeneracy and GAS follows.

The existence of a steady-state follows from Brouwer’s fixed point theorem since (13) evolves in a compact space (for a fixed \( N_r > 0 \)), and uniqueness follows from non-degeneracy and GAS. The positivity of the steady-state follows from persistence of the ORFM which can be shown graphically by the absence of critical siphons [19].