Translation in the cell under fierce competition for shared resources: a mathematical model

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During translation, mRNAs ‘compete’ for shared resources. Under stress conditions, during viral infection and also in high-throughput heterologous gene expression, these resources may become scarce, e.g. the pool of free ribosomes is starved, and then the competition may have a dramatic effect on the global dynamics of translation in the cell. We model this scenario using a network that includes $m$ ribosome flow models (RFMs) interconnected via a pool of free ribosomes. Each RFM models ribosome flow along an mRNA molecule, and the pool models the shared resource. We assume that the number of mRNAs is large, so many ribosomes are attached to the mRNAs, and the pool is starved. Our analysis shows that adding an mRNA has an intricate effect on the total protein production. The new mRNA produces new proteins, but the other mRNAs produce less proteins, as the pool that feeds these mRNAs now has a smaller abundance of ribosomes. As the number of mRNAs increases, the marginal utility of adding another mRNA diminishes, and the total protein production rate saturates to a limiting value. We demonstrate our approach using an example of insulin protein production in a cell-free system.

1. Introduction

mRNA translation is a fundamental process in gene expression, i.e. the transformation of genetic information into a functional protein [1]. During translation, the ribosomes (complex macro-molecules) scan the mRNA in a sequential manner, ‘read’ it codon by codon, and build a corresponding chain of amino acids. When the ribosome reaches a stop codon, it detaches from the mRNA and releases the chain that, after additional processing, becomes a functional protein.

Many ribosomes may attach to the same mRNA and translate it in parallel. This pipelining increases the protein production rate. The speed of the ribosome movement along the mRNA is determined by the mRNA sequence and structure, pauses due to collisions with other ribosomes, and by various translation factors, e.g. the abundance of cognate tRNA molecules in the vicinity of the mRNA. Understanding the dynamics of translation is of considerable importance, as it plays a major role in determining the protein production rate [2,3]. Furthermore, the dynamics of mRNA translation and specifically the evolution of ribosomal ‘traffic jams’ [4,5] along an mRNA have been implicated to various diseases [6,7].

Computational models for ribosome flow along an mRNA and the resulting protein production rate can be used to integrate and explain the growing number of experimental findings (e.g. via methods like ribosome profiling [8] that can be performed for single cells [9], and methods for imaging the translation of a single mRNA molecule [10]). Such models can also predict the effect of various manipulations or regulation of the genetic machinery on the protein production rate. Such manipulations are common both in biotechnology and also during viral infection, where the virus ‘hijacks’ and potentially shuts...
down parts of the host cell translation machinery. For example, SARS-CoV-2 uses a multipronged strategy to impede host protein synthesis and affect translation in a global manner [11]. In addition, heterologous genes tend to overload the translation machinery and also have a global effect on translation [12,13].

A popular model for ribosome flow, and many other natural and artificial systems and processes, is the totally asymmetric simple exclusion process (TASEP) (see e.g. [14,15]). This is a stochastic discrete-time process that includes a one-dimensional chain of sites and particles that hop stochastically along the chain in a unidirectional manner. Simple exclusion refers to the fact that two particles cannot be in the same site at the same time, i.e. a particle can only hop to an empty site. In the context of translation, the particles are ribosomes and the sites are consecutive (groups of) codons along the mRNA [16–18]. Simple exclusion corresponds to the fact that a ribosome cannot ‘overtake’ a ribosome in front of it, as the information on the mRNA must be decoded in a sequential manner. TASEP has become a phenomenological model in statistical mechanics, yet rigorous analysis of this model is difficult, except for some very special cases, e.g. when all the internal hopping rates are assumed to be equal [14].

The ribosome flow model (RFM) is the dynamic mean-field approximation of TASEP. This yields a continuous-time, deterministic, nonlinear model for the flow of ribosomes along the mRNA [19]. The RFM is highly amenable to analysis using tools from systems and control theory, and it has been used to model and analyse many important aspects of translation including: entrainment of the protein production rate to periodic initiation and elongation rates with a common period [20], sensitivity analysis of the steady-state production rate [21], optimizing the protein production rate subject to convex constraints on the rates [22], the effect of ribosome recycling [23,24], determining the ribosome density that maximizes protein production [25], stochastic variability in translation [26], maximizing the average protein production [27] and more [28].

The cell is a factory for producing proteins that includes a large number of mRNA molecules and ribosomes. For example, a Saccharomyces cerevisiae cell includes about 60 000 mRNA molecules and 200 000 ribosomes. About 85% of the ribosomes are associated with mRNAs [29–31], and the rest form the ‘pool of free ribosomes’. The mRNAs thus indirectly ‘compete’ for the available ribosomes. This generates a network with intricate indirect coupling between the mRNAs. For example, ribosomal ‘traffic jams’ on mRNAs may deplete the pool leading to lower initiation rates in the other mRNAs. When the shared resources are abundant the coupling between mRNAs due to this competition is weak, and the network can potentially be analysed using models of translation along a single, isolated RNA. However, when the resources are scarce, e.g. when the pool of free ribosomes is starved, the competition may have a strong effect on the global dynamics of translation in the cell.

The latter scenario may be relevant both in synthetic systems, where the goal is to optimize the production rate, and under various physiological conditions. For example, under stress conditions or during a high-yield viral infection, where the viral mRNAs ‘hijack’ the translation machinery, and consume many of the shared resources to produce viral proteins. It may also be relevant in heterologous gene expression (e.g. when the heterologous gene is overexpressed and consumes most of the ribosomes in the cell), and in cell-free systems where the number of mRNA molecules may be relatively large in comparison with the number of ribosomes [32].

We study this scenario using a mathematical model that includes a network of m RFMs interconnected via a pool of free ribosomes. The RFM belongs to the class of compartmental models that have been used in various domains of systems biology including genetics, physiology and pharmacology [33]. Each RFM describes the dynamics of translation along one mRNA molecule, and the interconnection via the pool encapsulates the competition for shared resources. This model was first suggested in [34]. Mathematically, it is a cooperative dynamical system [35,36] that admits a first integral: the total density of ribosomes in the network is conserved. Such systems have a well-ordered asymptotic behaviour [37,38]. It was shown in [34] that any solution of the network converges to a steady state, where the flow of ribosomes into and out of any site along any mRNA is equal. Also, the flows in and out of the pool are equal. Sensitivity analysis of this steady state [34] with respect to modifying one of the translation rates in a specific mRNA demonstrated that the steady-state production rates in all the other mRNAs either increase or all decrease.

The model based on networks of RFMs has already been validated experimentally, and applied to predict the density of ribosomes along different mRNAs, the protein levels of different genes, and even for re-engineering ribosomal traffic jams (see e.g. [19,31]).

A generalization of this network, that includes the possibility of ribosome drop-off and attachment at each site along the RFM, was recently suggested in [39]. Simulations of this model showed that ribosome drop-off from an isolated mRNA always decreases the protein production rate, yet in the network ribosome drop-off from a jammed mRNA may increase the total production rate of all the mRNAs, as the drop-off frees ribosomes that enter the pool, and this improves the production rate in the other mRNAs. This illustrates how the network perspective provides new biological insights.

In this paper, we analyse this network of m RFMs interconnected via a pool from a new, structural perspective, that is, we study how adding new RFMs to the network affects the dynamics. The main contributions of this paper include the following:

1. We prove that adding an RFM to the network always decreases the steady-state pool density. This makes sense, as every new RFM ‘consumes’ ribosomes.
2. We show that adding an RFM has an intricate effect on the network steady state: on the one hand, the additional RFM produces new proteins. On the other hand, the other RFMs produce less proteins, as the pool that feeds these RFMs now has a smaller abundance of ribosomes.
3. We provide a detailed asymptotic analysis of the network steady state when the pool is starved. In particular, we show that in this case the initiation rates in every RFM become the bottleneck rates, and provide a closed-form expression for the total steady-state density and total production rate on any subset of RFMs in the network, relative to the steady-state pool density.
4. We provide an explicit bound for the total production rate of the network when the number of RFMs is very large. In particular, we show how the total density of ribosomes in the networks bounds the total production rate. This bound demonstrates that when the number of
RFMs increases, the marginal utility of adding another RFM diminishes, and the analysis of the protein production saturates to a limiting value.

(5) We demonstrate our analysis approach for the case of producing insulin proteins in a cell-free system, and show how it can provide useful guidelines for setting the parameters in such a system.

The remainder of this paper is organized as follows. The next section reviews the network model. Before going into the mathematical analysis, §3 illustrates several structural questions that can be studied using simulations of the model. Section 4 details the main mathematical results. Section 5 reports analyses of a biological system (gene expression in a cell-free system) and how it can provide useful guidelines for setting the parameters in such a system.

2. The mathematical model

We use a model that includes $m$ RFMs interconnected via a pool of free ribosomes. Each RFM models the dynamics of ribosome flow along one mRNA molecule and, in particular, each RFM may have a different length and different parameters (i.e. different codon decoding rates and initiation rates). The pool of free ribosomes represents ribosomes in the cell that are not attached to any mRNA. We begin by reviewing the various components of this network.

2.1. Ribosome flow model

The RFM includes $n$ state variables $x_i, \ldots, x_n$ representing the normalized ribosome density in $n$ sites along the mRNA, where each site corresponds to a group of consecutive codons. The density is normalized such that $x_i(t) \in [0, 1]$ for all $i$, where $x_i(t) = 0$ represents that the site is empty, and $x_i(t) = 1$ represents that the site is completely full. Thus, $x_i(t)$ may also be interpreted as the probability that site $i$ is occupied at time $t$. The RFM also includes $n + 1$ positive parameters $\lambda_0, \ldots, \lambda_n$ where $\lambda_i$ controls the transition rate from site $i$ to site $i + 1$. In particular $\lambda_0$ controls the initiation rate, and $\lambda_n$ controls the exit rate.

The dynamics of the RFM is described by $n$ balance equations,

$$
\begin{align*}
\dot{x}_1 &= \lambda_0 (1 - x_1) - \lambda_1 x_1 (1 - x_2), \\
\dot{x}_2 &= \lambda_1 x_1 (1 - x_2) - \lambda_2 x_2 (1 - x_3), \\
&\vdots \\
\dot{x}_n &= \lambda_{n-1} x_{n-1} (1 - x_n) - \lambda_n x_n.
\end{align*}
$$

And

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

To explain this, consider the equation for the change in density in the second site, namely,

$$
\dot{x}_2 = \lambda_1 x_1 (1 - x_2) - \lambda_2 x_2 (1 - x_3).
$$

The term $\lambda_1 x_1 (1 - x_2)$ represents the flow of ribosomes from site 1 to site 2. This is proportional to the transition rate $\lambda_1$, to the density of ribosomes $x_1$ in site 1 and to the ‘free space’ $(1 - x_2)$ in site 2. In particular, if site 2 fills up, i.e. $x_2$ is close to one, then the flow into site 2 decreases to zero. This is a ‘soft’ version of the simple exclusion principle, i.e. the notion that two particles cannot be in the same place at the same time. Similarly, the second term on the right-hand side of (2.2) is the flow from site 2 to site 3. Thus, equation (2.2) states that the change in density in site 2 is the flow from site 1 to site 2 minus the flow from site 2 to site 3. The exit rate from the last site is $R(t) = \lambda_n x_n(t)$, and this is also the protein production rate at time $t$ (see figure 1). Note that $x_i$ is dimensionless, and that $\lambda_i$ has units of 1/time. In all the biological simulations below, $\lambda_i$ is in units of 1/s.

The state space of the RFM is the $n$-dimensional cube $[0, 1]^n$. Since this invariant set is compact and convex, the RFM admits an equilibrium point $c \in [0, 1]^n$. At an equilibrium, the flows into each site and out of each site are equal, so all the site densities are constant. Analysis of the equations describing the equilibrium point shows that there is a single equilibrium point $c \in [0, 1]^n$ (see [40]).

The RFM has been extensively used for studying the translation of a single, isolated mRNA. The model is highly amenable to analysis using tools from systems and control theory. It was shown in [40] that the RFM is a totally positive differential system [41] and this implies that any solution of the RFM converges to the unique equilibrium $c$. In particular, the protein production rate $R(t) = \lambda_n x_n(t)$ converges to the steady-state production rate $R := \lambda_n c_n$. In other words, the positive transition rates $\lambda_0, \ldots, \lambda_n$ determine a unique steady-state density $x_1 = c_1, \ldots, x_n = c_n$ along the mRNA, and for any initial density the dynamics converges to this profile.

Poker et al. [22] derived a useful spectral representation for the mapping from the rates $\lambda_0, \ldots, \lambda_n$ to the steady-state $c$. Given the RFM, consider the $(n + 2) \times (n + 2)$ tri-diagonal matrix

$$
A := \begin{bmatrix}
0 & \lambda_0^{1/2} & 0 & 0 & \ldots & 0 & 0 & 0 \\
\lambda_0^{-1/2} & 0 & \lambda_1^{-1/2} & 0 & \ldots & 0 & 0 & 0 \\
0 & \lambda_1^{1/2} & 0 & \lambda_2^{-1/2} & \ldots & 0 & 0 & 0 \\
\vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\
0 & 0 & 0 & 0 & \ldots & \lambda_{n-1}^{-1/2} & 0 & \lambda_n^{1/2} \\
0 & 0 & 0 & 0 & \ldots & 0 & \lambda_n^{1/2} & 0
\end{bmatrix}
$$

Since $A$ is symmetric, all its eigenvalues are real. Since $A$ is an irreducible matrix, with all entries non-negative, the Perron–Frobenius theorem [42] implies that $A$ admits a simple maximal eigenvalue $\sigma > 0$, and the corresponding eigenvector $\zeta \in \mathbb{R}^{n+2}$ is unique (up to scaling) and satisfies $\zeta_i > 0$ for all $i \in \{1, \ldots, n + 2\}$. Then, the entries of $c$ satisfy [28]

$$
c_i = \frac{\zeta_i n}{\lambda_i^{1/2} \sigma^{n+1}}, \quad i = 1, \ldots, n.
$$
and the steady-state production rate satisfies
\[ R = \sigma^{-2}. \] (2.5)
In other words, the Perron eigenvalue and eigenvector of $A$ provide all the information needed to determine the steady-state profile $c$, and the steady-state production rate $R$ in the RFM.

This spectral representation has several implications. For example, it implies that it is possible to numerically calculate efficiently the steady state even for very large RFMs using algorithms for computing the Perron eigenvalue and eigenvector of tri-diagonal matrices. Also, it follows from (2.5) that the function $R = R(x_0, \ldots, x_n)$ is strictly concave [22], thus allowing to show that general steady-state protein production optimization problems are convex optimization problems [22]. It also reduces the sensitivity analysis of $R$ with respect to any rate $\lambda_i$ to an eigenvalue sensitivity problem for the matrix $A$ [21].

### 2.2. Ribosome flow model with input and output

As noted above, a cell typically includes a large number of mRNA molecules and ribosomes, that compete for shared resources, and studying translation on a single, isolated mRNA may thus provide limited insight on large-scale translation in the cell. To model translation in the cell requires a network of interconnected RFMs. The first step in building such a network is adding an input and output to the RFM. This yields the ribosome flow model with input and output (RFMIO) [34]. The RFMIO dynamics is
\[
\begin{align*}
\dot{x}_1 &= \lambda_0 (1 - x_1) - \lambda_1 x_1 (1 - x_2), \\
\dot{x}_2 &= \lambda_1 x_1 (1 - x_2) - \lambda_2 x_2 (1 - x_3), \\
&\quad \vdots \\
\dot{x}_n &= \lambda_{n-1} x_{n-1} (1 - x_n) - \lambda_n x_n \\
\end{align*}
\] (2.6)

The scalar input $u : \mathbb{R}_+ \rightarrow \mathbb{R}_+$ represents the density of ribosomes in the vicinity of the initiation site. Thus, if $u(t)$ is large then the effective initiation rate at time $t$, given by $u(t)\lambda_0$, increases. The scalar output $y(t) = \lambda_i x_i(t)$ is the rate of ribosomes exiting the mRNA at time $t$. The additional input and output allow to connect RFMIOs in a network. Note that for $u(t)\equiv 1$, equation (2.6) reduces to the RFM.

#### 2.3. The network

Raveh et al. [34] introduced a model composed of $m$ RFMIOs interconnected via a pool of free ribosomes (see figure 2). Let $n_i$, $i = 1, \ldots, m$, denote the length of RFMIO $i$. The state variables in RFMIO $i$ are denoted by $x_{i1}, \ldots, x_{in_i}$. The density in the pool at time $t$ is modelled by the scalar function $z(t)$. The ribosomes that initiate translation in RFMIO $i$ are supplied from the pool through the pool output function $G_i(z(t))$. Thus, the input to RFMIO $i$ is $u_i(t) = G_i(z(t))$, so the effective initiation rate in RFMIO $i$ is $\lambda_i G_i(z(t))$.

The functions $G_i : \mathbb{R}_+ \rightarrow \mathbb{R}_+$ satisfy $G_i(0) = 0$ (i.e. when the pool is empty the initiation rate in the RFMIO is zero), and $G_i(z)$ is continuous and strictly increasing in $z$ (i.e. an increase in the pool density yields an increase in the initiation rates). Many possible functions satisfy these constraints, e.g. $G_i(z) = cz$, with $c > 0$, and the uniformly bounded function $G_i(z) = \alpha \tanh (\beta z)$, with $\alpha, \beta > 0$.

The pool feeds all the RFMIOs, and is fed by the ribosomes exiting all the RFMIOs, so the balance equation for the change in $z(t)$ is
\[
\dot{z} = \sum_{i=1}^{m} y_i - \sum_{i=1}^{m} \lambda_i z_i(1 - x_i),
\] (2.7)

where $y_i$ is the ribosome exit rate from RFMIO $i$.

Let
\[
s(t) := z(t) + \sum_{i=1}^{m} \sum_{j=1}^{n_i} x_{ij}(t) \] (2.8)
denote the total density of ribosomes in the network at time $t$. Since ribosomes cannot leave nor enter the network,
\[
s(t) = s(0) \quad \text{for all } t \geq 0.
\] (2.9)

In other words, $s(t)$ is a first integral of the dynamics.

It is important to note that there is no direct link between the RFMIOs in the network, and that the competition is not ‘encoded’ by changing the dynamical equations as was done in other context-aware models (see e.g. [43,44]). Competition arises only due to the interconnection via the shared pool of free ribosomes.

Raveh et al. [34] used results on cooperative systems with a first integral (see e.g. [37,38]) to prove that any solution of the network converges to a steady state. More precisely, for any $p \geq 0$, let $L_p$ denote the $p$ level set of the first integral,
that is, $L_p$ includes all the initial conditions of pool and RFMIO densities with total density $s(0) = p$. Then $L_p$ includes a single equilibrium point, and any trajectory emanating from an initial condition in $L_p$ converges to this equilibrium point. In other words, any two initial conditions of the network with the same total ribosome density will converge to the same equilibrium state. At this state, the total density is distributed along the mRNAs and the pool such that the flow into and out of each site is equal.

Let $c_e \in [0, s(0)]$ denote the steady-state pool density, and let $c_{ej}$ denote the steady-state density in site $j$ in RFMIO #i. Also, let

$$
ez \left[ c_{e1} \ e_{e1} \cdots e_{e1} e_{e2} \cdots e_{en} \right]^T, \quad (2.10)$$

i.e. $c$ collects all the steady-state values in the network.

Here, we use the same network model as in [34] to study a different problem, namely, the effect of competition between multiple RFMIOs for scarce shared resources on the global dynamics of translation in the cell.

3. Simulation results

We begin with several synthetic simulation results that demonstrate the wealth of biological questions that can be addressed using the network model when allowing the number of RFMIOs $m$ to vary, that is, when mRNAs are added or removed from the network. This also illustrates our general analysis approach that combines the spectral representation of the steady state in every RFMIO with the equation describing the first integral of the network.

We begin by considering a network that includes $m$ identical RFMIOs, where each RFMIO has length $n_i = 2$, $i = 1, \ldots, m$. We also assume that every RFMIO is homogeneous, with $\lambda_0 = \lambda_1 = \lambda_m = 1$. (Note, however, that all the theoretical results in §4 below hold for general lengths and rates.) We also assume that $G_i(z) = z$ for all $i$ (i.e. the effective initiation rate is proportional to the number of free ribosomes in the pool).

To apply the spectral approach to each RFMIO in the network, let

$$A(c) := \begin{bmatrix} 0 & c & 0 & 0 \\ c & 1 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}, \quad (3.1)$$

where $c := c_e^{-1/2}$. Note that this is exactly the matrix (2.3) with $n = 2$, $\lambda_0 G(c) = c_1$, and $\lambda_1 = 1$ for $i = 1, 2$. The Perron root of $A(c)$ is

$$\sigma(c) = \frac{\sqrt{\sqrt{c_1 + 4} + c_1^2 + 2}}{\sqrt{2}}.$$

and the corresponding Perron eigenvector is

$$\xi(c) = \begin{bmatrix} (\sqrt{c_1 + 4} + 2 - 2)\sqrt{\sqrt{c_1 + 4} + 1} \\ 1/2(\sqrt{c_1 + 4} + c_1) \sqrt{\sqrt{c_1 + 4} + 1} \end{bmatrix}^T.$$

It follows from (2.4) that the steady-state densities in every RFMIO are

$$c_1(c) = \frac{2}{\sqrt{c_1 + 4} + c_1^2}$$

and

$$c_2(c) = \frac{2}{\sqrt{c_1 + 4} + c_1^2 + 2}, \quad (3.2)$$

and since $\lambda_2 = 1$, the steady-state production rate of each RFMIO is

$$R(c) = c_2(c).$$

The equation for the total density of ribosomes $s$ in the network is

$$s = c_e + m(c_1(c) + c_2(c)) = e^{-2} + m(c_1(c) + c_2(c)). \quad (3.3)$$

Combining this with (3.2) provides an explicit expression for $c$ as a function of $s$. This can be inverted (at least numerically) to conclude for every total density $s$ the corresponding $c$ (and thus $c_e$), and then the spectral approach allows to obtain all the steady-state profiles in all the RFMIOs.

The network allows to study how important steady-state quantities depend on the number of RFMIOs in the network. We first define several such quantities. The ratio between the density of ribosomes in the pool and the total density of ribosomes in the network is

$$q := \frac{c_e}{s}.$$ 

The total protein production at steady state, denoted TPR, is the production rate of all the RFMIOs in the network. Since there are $m$ identical RFMIOs,

$$TPR = m e_2(c).$$

As $m$ is increased, more ribosomes are attached to mRNAs and thus we can expect the steady-state pool density $c_e$ to go to zero. Then the initiation rate $\lambda_0 G(c) = c_1$ in each RFMIO becomes the bottleneck rate, and thus $c_1 \approx c_e$ for $i = 1, 2$, in every RFMIO. Substituting this in (3.3) gives $c_e \approx s/(1 + 2m)$, and the total production rate is then

$$TPR = m e_2 \approx m e_2 \approx \frac{ms}{1 + 2m}. \quad (3.4)$$

Thus, for a large $m$ we expect the total steady-state production rate in the network to converge to $s/2$.

Figure 3 depicts the exact network steady-state values for $s = 50$ as a function of the number of RFMIOs $m$. It may be seen that: (i) the steady-state densities $c_1$ and $c_2$ in every RFMIO decrease monotonically with $m$. The same holds for $q = c_e/s$, and (ii) the total production rate $m e_2$ increases monotonically with $m$, and converges to a saturation value of $s/2 = 25$.

Summarizing, the addition of mRNAs increases the total protein production, as ribosomes now also translate the new mRNAs. However, it decreases the translation rate of the other mRNAs by depleting the pool of free ribosomes. As the number of mRNAs increases, the marginal utility in adding another mRNA decreases to zero. The total production rate in the network is bounded, and an important factor in this bound is the total density of ribosomes in the network.

These simulation results suggest that when optimizing the production rate of a synthetic system (e.g. in high-throughput heterologous gene expression) it may be useful to use mRNA levels below the regime where the marginal utility in adding another mRNA becomes negligible.

The next section provides a rigorous analysis of these topics.
number of RFMIOs
pool output function, and
steady state
total ribosome density $s$

We begin by considering the effect of adding an RFMIO to
the network. We introduce some notation. Recall that
RFMIO #1 is characterized by the tuple
\[ T_1 := \{ n_i, G_i, \lambda_{i,0}, \ldots, \lambda_{i,n_i} \}, \]
where $n_i$ is the length of RFMIO #1, $G_i : \mathbb{R}_+ \to \mathbb{R}_+$ is the $i$th pool output function, and $\lambda_i$ are the rates along RFMIO #1.

Consider a network with $m+1$ RFMIOs obtained by adding an RFMIO to a network of $m$ RFMIOs. Let $e_i(m)$ denote the pool density in the network with $m$ RFMIOs. What is the relation between the steady-state pool densities before and after adding RFMIO #$(m+1)$? The next result shows that $e_i(m+1)$ is always smaller than $e_i(m)$ (cf. figure 3a).

**Proposition 4.1.** Fix $s > 0$, and a collection of RFMIOs $T_1, T_2, \ldots$. For any $m$, consider a network of $m$ RFMIOs $T_1, \ldots, T_m$ interconnected via a pool of free ribosomes, with total ribosome density $s$. Let $(e(m))$ denote the corresponding network steady state (see (2.10)), where the coordinates depend on $m$. Then
\[ 0 < e_i(m+1) < e_i(m). \]

The proof is placed in appendix A.1.

In other words, adding an RFMIO to the network, while keeping the total ribosome density constant, always decreases the steady-state pool density. This makes sense, as the new mRNA ‘consumes’ ribosomes from the pool.

Proposition 4.1 implies in particular that in a network built by repeatedly adding new RFMIOs the sequence of steady-state pool densities $e_i(1), e_i(2), \ldots$ is monotonically decreasing. Since $e_i(m) \geq 0$ for all $m$, this implies that the limit
\[ \lim_{m \to \infty} e_i(m) \]
exists. The next result shows that this limit is zero. Since we take $m \to \infty$, we need to impose some technical conditions on the RFMIOs.

**Assumption 4.2.** From here on we always assume that the following properties hold.

1. There exists $\lambda_* > 0$ such that
\[ \lambda^*_i \geq \lambda_* \quad \text{for all } i, \]
i.e. all the initiation rates are bounded from below by $\lambda_*$.\[ \lambda^*_i \leq \lambda^* \quad \text{for all } i, \]
i.e. all the exit rates are bounded from above by $\lambda^*$.
2. There exist $p > 0$ and $g_* > 0$ such that for any $z \in [0, p]$, we have
\[ G_i(z) \geq g_* \quad \text{for all } i, \]
i.e. all the pool output functions $G_i(z)$ are bounded from below by the linear function $g_* z$ on the interval $[0, p]$.

These three conditions are clearly reasonable. The next result shows that under these conditions, the limit in (4.2) is zero (cf. figure 3a).

**Proposition 4.3.** Fix $s > 0$, and a collection of RFMIOs $T_1, T_2, \ldots$. For any $m$, consider a network of $m$ RFMIOs $T_1, \ldots, T_m$ interconnected via a pool of free ribosomes, with total ribosome density $s$. Let $(e(m))$ denote the corresponding network steady state (see (2.10)), with coordinates that depend on $m$. Then
\[ \lim_{m \to \infty} e_i(m) = 0. \]

The proof is placed in appendix A.2.

From a biological point of view, the case $m \to \infty$ may seem unreasonable. However, Proposition 4.3 implies that for a large $m$, $e_i(m)$ will be small. This scenario is the focus of this paper. Indeed, when the pool includes a large number of free ribosomes there is little competition, and thus the indirect coupling between the mRNAs is weak. The interesting case is thus when the pool is close to being depleted, that is, when $e_i$ is small. In the biological context, this may represent a cell under stress conditions, or under a high-yield viral infection, or when there are many ribosomal ‘traffic jams’ along the mRNAs, so the pool is depleted.

The next result uses the spectral representation to analyse the steady-state densities and production rate in every RFMIO when the pool is starved.

**Figure 3.** (a) Steady states $e_1$, $e_2$ and $e_3$ as a function of the number of RFMIOs $m$ when $s = 50$; (b) total production rate $me_2$ in the network as a function of the number of RFMIOs $m$. 4. Main results
Proposition 4.4. Consider a network of RFMIOs $T_1, T_2, \ldots$, interconnected via a pool. Suppose that either the total ribosome density $s$ goes to zero, or that the number of RFMIOs tends to infinity, so that $e_z \to 0$. Then for any $i$, RFMIO $\#i$ satisfies
\[ \lim_{e_z \to 0} \frac{e^i_j}{G_i(e_z)} = \frac{\lambda^0_i}{\lambda^j_i}, \quad j = 1, \ldots, n_i. \]  
(4.4)

In particular, if the pool output function $G_i$ is differentiable at zero, then
\[ \lim_{e_z \to 0} \frac{e^i_j}{e_z} = \frac{\lambda^0_j G_i(0)}{\lambda^j_i}, \quad j = 1, \ldots, n_i. \]  
(4.5)

The proof is placed in appendix A.3.

In other words, when the pool becomes depleted (e.g. because $m$ is large or the total ribosome density $s$ is small) every density along the $i$th mRNA behaves asymptotically like the pool output function $G_i(e_z)$. This makes sense, as the effective initiation rate $\lambda^0_i / \lambda^j_i$ becomes the bottleneck rate in the mRNA. Note that (4.4) implies that $e^i_j / \lambda^0_i G_i(e_z)$ is inversely proportional to $\lambda^j_i$. This is reasonable, as $\lambda^j_i$ controls the flow out of site $j$.

Example 4.5. To illustrate proposition 4.4, we use a network consisting of $m$ identical RFMIOs. Each RFMIO has length 5 and rates
\[ [0.1678, 0.2572, 0.2758, 0.2514, 0.2612, 0.3002]. \]  
(4.6)

These values are taken from Zarai et al. [45] and correspond to the $S.\ cer\ce{e}\v R\ce{e} \ce{es}i\ce{ae}i$ gene YBL025W that encodes the protein RNR10, which is related to regulation of RNA polymerase I. This gene has 145 codons (excluding the stop codon), and was divided into six consecutive groups of codons: the first group includes the first 24 codons (that are also related to later stages of initiation). The other groups include 25 non-overlapping codons each, except for the last one that includes 21 codons. This partitioning was found to optimize the correlation between the RFM prediction and biological data measurements (see [45] for more details). We increased $m$ from 1 to 1000 while keeping the total ribosome density fixed at $s = 20$, thus depleting the steady-state pool density. The input functions are $G_i(x) = x$ for all $i$. Since all the RFMIOs are identical, it is sufficient to consider the steady-state density in a single RFMIO. Figure 4 depicts $e_j/e_z$, $j = 1, \ldots, 5$, as a function of $1/e_z$. It may be seen that as $e_z$ decreases, every ratio $e_j/e_z$ converges to the asymptotic value given in proposition 4.4. The following result is an immediate corollary of proposition 4.4. Recall that the constant total ribosome density in the network is
\[ s = e_z + \sum_{i=1}^m \sum_{j=1}^{n_i} e^i_j, \]  
(4.7)

and the total steady-state production rate is
\[ \text{TPR} := \sum_{i=1}^m \lambda^0_i e^i_{n_i}. \]

Also, let $q := e_z / s$ denote the ratio between the free ribosomes and the total number of ribosomes in the network.

**Corollary 4.6.** Suppose that the pool output functions $G_i$ are differentiable at zero for all $i = 1, 2, \ldots$. Then,

1. The total production rate satisfies
\[ \lim_{e_z \to 0} \frac{\text{TPR}}{e_z} = \sum_{i=1}^m \lambda^0_i G_i(0). \]  
(4.8)

2. The ratio between the free ribosomes to the total number of ribosomes in the network satisfies
\[ \lim_{e_z \to 0} q = \left(1 + \sum_{i=1}^m \frac{\lambda^0_i G_i(0) n_i}{H(\lambda^1_i, \ldots, \lambda^4_i)}\right)^{-1}, \]  
(4.9)

where $H(\lambda^1_i, \ldots, \lambda^4_i) := n_i (\sum_{j=1}^5 (1/\lambda^j_i))^{-1}$ is the harmonic mean of the rates $\lambda^1_i, \ldots, \lambda^4_i$, that is, all the rates except for the initiation rate.

The proof is placed in appendix A.4. These results provide closed-form asymptotic expressions for important biological quantities when the pool is starved. Note that as $e_z \to 0$, TPR/$e_z$ depends on all the initiation rates $\lambda^0_i$, but not on any of the other rates (since the number of ribosomes along any mRNA is low, there are no traffic jams). However, the ratio between the density of ribosomes in the pool and the total number of ribosomes in the network does depend on the harmonic mean of all the rates in the network.

We note that a similar closed-form expression can be obtained for the total steady-state density and total production rate on any subset of RFMIOs in the network.

Example 4.7. Consider again the example in §3. In this case, $n_i = 2$, $\lambda^0_1 = \lambda^2_1 = \lambda^2_2 = 1$, and $G_i(x) = x$ for all $i$, so corollary 4.6 implies that
\[ \lim_{e_z \to 0} \frac{\text{TPR}}{e_z} = m \]  
and
\[ \lim_{e_z \to 0} q = (1 + 2m)^{-1}. \]

This shows in particular that the estimates in (3.4) are actually exact when $e_z \to 0$. The following result is an immediate corollary of proposition 4.4. Recall that the constant total ribosome density in the network is
\[ s = e_z + \sum_{i=1}^m \sum_{j=1}^{n_i} e^i_j, \]  
(4.7)

and the total steady-state production rate is
\[ \text{TPR} := \sum_{i=1}^m \lambda^0_i e^i_{n_i}. \]

Also, let $q := e_z / s$ denote the ratio between the free ribosomes and the total number of ribosomes in the network.
Corollary 4.6 implies that when the pool is starved we can replace an entire network of $m$ identical RFMIOs by a much simpler network, while keeping the asymptotic steady-state properties unchanged.

**Proposition 4.8.** Let $s > 0$ denote the total number of ribosomes. Consider the following two networks of RFMIOs:

1. A network of $m$ identical RFMIOs, each of length $n$, rates $\lambda_0, \ldots, \lambda_n$, and output functions $G(z) = g z$, with $g > 0$. Let $\text{TPR}[q]$ denote the total production rate [ratio of free ribosomes and s] in this network.

2. A network consisting of a single RFMIO of length $\lambda_0$, $\lambda_1$, and $G(z) = g z$, with $g > 0$. Let $\text{TPR}[q]$ denote the total production rate [ratio between free ribosomes and s] in this network.

If the parameters of the second network are chosen such that

$$\bar{\lambda}_0 g = \lambda_0 g m$$

and

$$(\bar{\lambda}_1)^{-1} = \sum_{i=1}^{n} (\lambda_i)^{-1},$$

then, as $s \to 0$

$$\lim_{c_0 \to 0} \frac{\text{TPR}}{c_0} = \lim_{c_1 \to 0} \frac{\text{TPR}}{c_1}$$

and

$$\lim_{\epsilon_1 \to 0} q = \lim_{\epsilon_1 \to 0} \frac{\text{TPR}}{c_1}.$$

The proof is placed in appendix A.5.

In other words, we can use a network with a pool and a single RFMIO, with a single site, to simulate and analyse the first network. Note that conditions (4.10) and (4.11) are quite intuitive. Roughly speaking, the first implies that the initiation rates in the two networks are equal (taking into account that in the first network there are $m$ RFMIOs and in the second a single RFMIO), whereas the second condition requires that some mean of the other rates along the RFMIO is also equal.

**Example 4.9.** Consider again the network in example 4.5. Recall that this has $m$ identical RFMIOs of length $n = 5$ and the rates given in (4.6). In this case, $g = 1$, $\lambda_0 = 0.1678$ and

$$\sum_{i=1}^{5} (\lambda_i)^{-1} = 18.6512.$$ 

Proposition 4.8 implies that we can replace this network of $m$ RFMIOs by a network consisting of a single RFMIO of length one, with $G(x) = x$, $\lambda_0 = 0.1678 m$ and $\bar{\lambda}_1 = 1/18.6512$, and the asymptotic behaviour of the two networks when the pool is starved will be identical.

5. A biological example: production of insulin in a cell-free system

In this section, we apply our model to analyse the production of insulin protein in a cell-free system. A cell-free system is an in vitro-based approach to study and/or generate biological reactions that take place within cells via the isolation of relevant cellular components (e.g. ribosomes, RNA polymerases, tRNA molecules). This approach reduces the complex interactions typically found when working with whole cells. Cell-free systems are often used in biotechnology for heterologous gene expression [46].

Data on the coding region of insulin fitted to *S. cerevisiae* was taken from Kjeldsen [47]. The codon decoding rates, that are based on the analysis of typical decoding rates in vivo from ribo-seq data [48], were taken from Dana & Tuller [49]. Groups of 10 consecutive codons were coarse grained into one RFMIO site, as was done in previous studies (see e.g. [31]). This yields 12 sites. The transition rate $\lambda_i$ of site $i$ is the inverse of the sum of the decoding times along the 10 related codons. These rates were then normalized such that their average value is 10 codons per second (the typical decoding rate in eukaryotes [50]).

Since we study the translation of insulin in a cell-free system, we assume that all the mRNAAs in the network encode the protein insulin. Thus, the network includes $m$ identical RFMIOs interconnected via a pool.

We set $s = 25 \times 10^4$, $m = 39.5 \times 10^3$ (see the data in [31,51,52]) and assume that all the pool output functions are identical: $G_j(x) = c x$, for all $i = 1, \ldots, m$. To calibrate the constant $c$, we assume that $c$ maximizes the effective initiation rate while keeping it lower than all the other transition rates. Mathematically, this yields the equation

$$\lambda_0 G(z) = \lambda_0 c z = \min\{\lambda_1, \ldots, \lambda_0\},$$

and this gives $c = 7.4758 \times 10^{-5}$.

To study the scenario where the pool is starved, we consider two cases: in the first we fix $m$ and decrease $s$, and in the second we fix $s$ and increase $m$. From a biological perspective, both cases correspond to the fact that ribosomes may be ‘more expensive’ then mRNAs, and the goal is to optimize production while using a minimal number of ribosomes.

5.1. Varying the total density of ribosomes

Consider the case where $m = 39.5 \times 10^3$ is fixed, and $s$ varies. Figure 5 depicts $\text{TPR}/c$, (that is, the ratio between the steady-state total production rate and the steady-state pool density) as a function of $m/s$ (that is, the number of mRNA molecules divided by the total number of ribosomes in the network). As
expected, TPR/c decreases with m/s, and converges, as s → 0, to the asymptotic value \( \sum_{s=1}^{m} \lambda_s G_s(0) = cm_{0} = 29.56 \).

5.2. Varying the number of mRNAs

Consider the case where s = 25 × 10⁴ is fixed, and m is varied. Figure 6 depicts the number of free ribosomes c as a function of m/s. As m increases, c decreases sharply, as more ribosomes attach to the additional mRNAs, and thus the pool is starved. In particular, c goes to zero as m → \infty (see proposition 4.3). In practice, when m/s = 80 we already get a very low value of c, and then the asymptotic results described in our analysis can already be used.

Note that these results can provide important guidelines for setting the cell-free system parameters. For example, figure 5 shows that to achieve a steady-state production rate that is 99% of the maximal possible production rate we should set m/s = 10, that is, 10 mRNA molecules for each ribosome in the system. Our model allows to estimate the total production rate for any m/s value.

6. Discussion

The competition for shared resources plays an important role in gene expression. It is known that competition for free RNPs and free ribosomal subunits is a major bottleneck for gene expression in bacteria [53]. Competition for shared resources also hampers our ability to reliably predict the behaviour of synthetic biology constructs (see [13,43,54,55] and references therein).

We considered a network of mRNAs fed by a pool of free ribosomes in the scenario when the pool is starved. This scenario is expected to be relevant for example in biological networks under stress conditions or viral attack, and in synthetic networks designed to optimize the total production rate.

We used a mathematical model of a network of RFMIOs connected via a pool of free ribosomes. Using the spectral representation of the RFMIO steady state we derived closed-form expressions for several relevant biological quantities in the regime when the pool is starved. These include the total protein production rate in the network, and the ratio between the number of ribosomes in the pool and the total number of ribosomes in the network.

We demonstrated the analytical results using both synthetic examples and an example based on biological data. The results reported here can be used both in systems biology studies of natural systems and for the design of synthetic constructs. We believe that an interesting direction for further research is to use our results in the biological context of a cell attacked by viruses. This will allow to study both qualitatively and quantitatively important questions. For example, can the virus effectively shut down the host protein production (and in particular immune-related proteins) by depleting the pool of free ribosomes or are other mechanisms needed?

Currently, there are no systematic measurements of the dynamic ratio between the number of free ribosomes and the number of mRNAs in cells. Nevertheless, stress conditions are known to induce ribosomal traffic jams and thus we may expect a significant reduction in the number of free ribosomes. Even in the presence of physiological feedback on the number of ribosomes and mRNAs in such conditions, production of new ribosomes may be slow as it consumes considerable cellular resources. Novel experimental procedures are needed to study these issues in the cell under various conditions.

Data accessibility. This article has no additional data.

Authors’ contributions. R.K.: writing—original draft; E.A.: writing—original draft; T.T.: writing—original draft; M.M.: writing—original draft.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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Appendix A. Proofs

For the sake of readability, we begin with a short and general description of our analysis approach. Consider without loss of generality the steady-state densities e = [e₁, ..., eₙ] along RFMIO #1 in the network. The spectral representation implies that we can retrieve e from the Perron root and Perron eigenvector of the matrix

\[
A_{1} := \begin{bmatrix}
0 & (\lambda_{1}^{1})^{-1/2} & 0 & \cdots & 0 \\
(\lambda_{1}^{1})^{1/2} & 0 & (\lambda_{1}^{1})^{-1/2} & \cdots & 0 \\
0 & (\lambda_{1}^{1})^{-1/2} & 0 & \cdots & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
0 & 0 & 0 & \cdots & (\lambda_{n}^{1})^{-1/2}
\end{bmatrix}
\]

Endnote

^The final result of this process are the rates: 10.01, 10.33, 10.25, 9.97, 10.67, 10.59, 9.40, 9.97, 9.75, 10.67, 10.23, 10.13, 8.03.
Indeed, at steady state the pool density is \( z(t) = e_{c_0} \), so the initiation rate at RFMIO \( #1 \) is \( \lambda_0 G_1(z(t)) = \lambda_0 G_1(e_{c_0}) \).

When \( c_0 \) is close to zero, so is \( G_1(e_{c_0}) \) and this implies that entries \((1, 2)\) and \((2, 1)\) in \( A \) are very large. We use an asymptotic analysis of the spectral properties of \( A \) to derive an approximate expression for \( e^c \) and, similarly, for any \( e^i, i = 1, \ldots, m \). Now by (2.8) and (2.9),

\[
e^c = s - \sum_{j=1}^m \sum_{j=1}^n e_{ij}^c,
\]

and thus we obtain the entire steady state of the network.

We begin with several auxiliary results that describe the asymptotic spectral properties of a specific tri-diagonal matrix. We use \( \mathbb{R}_+^n := \{ x \in \mathbb{R}^n \, | \, x_i \geq 0, \, i = 1, 2, \ldots, n \} \) to denote the non-negative orthant in \( \mathbb{R}^n \), and \( \mathbb{R}_{++}^n := \{ x \in \mathbb{R}^n \, | x_i > 0, \, i = 1, 2, \ldots, n \} \) to denote the positive orthant in \( \mathbb{R}^n \). For a Hermitian matrix \( S \in \mathbb{C}^{N \times N} \) we denote its eigenvalues by

\[
\sigma_i(S) \geq \cdots \geq \sigma_N(S).
\]

**Theorem A.1.** Given \( c > 0 \) and a vector \( \alpha = [\alpha_1, \ldots, \alpha_N]_T \) with \( \alpha_i > 0 \), consider the \( N \times N \) tri-diagonal and symmetric matrix

\[
T[c, \alpha] := \begin{bmatrix}
0 & c & 0 & \cdots & 0 & 0 & 0 \\
c & 0 & \alpha_1 & \cdots & 0 & 0 & 0 \\
0 & \alpha_1 & 0 & \cdots & 0 & 0 & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\
0 & 0 & 0 & \cdots & \alpha_{N-3} & 0 & \alpha_{N-2} \\
0 & 0 & 0 & \cdots & 0 & \alpha_{N-2} & 0
\end{bmatrix}
\]

(A1)

Let \( M := \max_{1 \leq i \leq N} \alpha_i \). Then for any \( c \geq 2M \), we have

\[
\sigma_i(T[c, \alpha]) \in [c - 2M, c + M]
\]

and

\[
\lim_{c \to \infty} \frac{1}{c} \sigma_i(T[c, \alpha]) = 1.
\]

(A3)

Furthermore, let \( \zeta(c) \in \mathbb{R}_{++}^N \) be the eigenvector of \( T[c, \alpha] \) corresponding to \( \sigma_1(T[c, \alpha]) \), normalized such that its first entry is \( \zeta_1(c) = 1 \). Then

\[
\lim_{c \to \infty} \zeta(c) = [1, \, 1, \, 0, \ldots, \, 0]^T.
\]

(A4)

More precisely, the entries of this vector satisfy

\[
\lim_{c \to \infty} \frac{\zeta_{i+1}(c)}{\zeta_i(c)} = \alpha_i, \quad \text{for all } i \in \{1, \ldots, N - 2\}.
\]

(A5)

**Proof.** Recall that a theorem of Weyl [42, Section 4.3] asserts that if \( A, B \in \mathbb{C}^{N \times N} \) are Hermitian then for any \( i, j \in \{1, \ldots, N\} \), we have

\[
\sigma_{i+j-N}(A + B) \leq \sigma_i(A) + \sigma_j(B), \quad i + j \leq N + 1
\]

and

\[
\sigma_j(A) + \sigma_j(B) \leq \sigma_{i+j-N}(A + B), \quad i + j \geq N + 1.
\]

(A6)

Recall also that if \( | \cdot : \mathbb{C}^N \to \mathbb{R}_+ \) is a vector norm, and \( \| \cdot \| : \mathbb{C}^{N \times N} \to \mathbb{R}_+ \) is the induced matrix norm, then \( |\sigma_j(S)| \leq \|S\| \) for any \( i \).

We can now prove theorem A.1. First note that for any \( c \geq M \), we have

\[
\sigma_i(T[c, \alpha]) \leq \|T[c, \alpha]\|_\infty \leq M + c,
\]

and this proves the upper bound in (A 2).

To prove the lower bound, fix \( c > 0 \). Define \( A := T[c, 0, \ldots, 0], \, B := T[0, \alpha] \). Note that \( A + B = T[c, \alpha] \). Applying (A 6) with \( i = 1 \) and \( j = N \) gives

\[
\sigma_i(T[c, \alpha]) \geq \sigma_i(T[c, 0, \ldots, 0]) + \sigma_N(T[0, \alpha]) = c + \sigma_N(T[0, \alpha]) \geq c - 2M,
\]

where the last inequality follows from the fact that

\[
\|\sigma_N(T[0, \alpha])\| \leq \|T[0, \alpha]\|_\infty \leq \max_{1 \leq i \leq N-3} \{\alpha_i + \alpha_{i+1}\} \leq 2M.
\]

This completes the proof of (A 2). Taking \( c \to \infty \) in (A 2) proves (A 3).

To prove (A 4), let \( 0 < c_1 < c_2 < \ldots \) be such that \( \lim_{k \to \infty} c_k = \infty \). To simplify the notation, let \( \zeta^k := \zeta(c_k) \in \mathbb{R}_{++}^N \). We may assume that every \( \zeta^k \) has norm one, and thus we can extract a subsequence \( \zeta^k \), \( k = 1, 2, \ldots \), that converges to a limit vector \( \zeta \in \mathbb{R}_{++}^N \), that also has norm one. Then

\[
\zeta = \lim_{k \to \infty} \zeta^k = \lim_{j \to \infty} \sigma_j(T[c_j, \alpha_j]) = T[1, 0, \ldots, 0] \zeta.
\]

(A7)

Thus, \( \zeta \) is a normalized eigenvector of \( T[1, 0, \ldots, 0] \) corresponding to \( \sigma_1(T[1, 0, \ldots, 0]) = 1 \), and it is straightforward to verify that

\[
\zeta = \frac{1}{\sqrt{2}} [1 \, 1 \, 0 \ldots \, 0]^T.
\]

A direct calculation gives

\[
D D^T = c S(c),
\]

where

\[
S(c) = \begin{bmatrix}
0 & 1 & 0 & 0 & \cdots & 0 & 0 & 0 \\
1 & 0 & \alpha_1 & 0 & \cdots & 0 & 0 & 0 \\
0 & \alpha_1 & 0 & \alpha_2 & \cdots & 0 & 0 & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \vdots \\
0 & 0 & 0 & \cdots & \alpha_{N-3} & 0 & \alpha_{N-2} & 0 \\
0 & 0 & 0 & \cdots & 0 & \alpha_{N-2} & 0 & 0
\end{bmatrix}
\]

(A8)

Let

\[
S(c) := D \zeta(c) = \begin{bmatrix}
\zeta_1(c) & \zeta_2(c) & c \zeta_3(c) & \cdots & c^{N-2} \zeta_N(c)
\end{bmatrix}^T.
\]

(A9)

\[
= [\zeta_1(c) \, \zeta_2(c) \, c \zeta_3(c) \, \cdots \, c^{N-2} \zeta_N(c)]^T.
\]

(A10)
Then
\[ T(c)\xi(c) = \sigma(c)\xi(c) \iff S(c)v(c) = \left(\frac{\sigma(c)}{c}\right)v(c). \tag{A11} \]
In particular
\[ \text{spec}(S(c)) = \frac{1}{c} \text{spec}(T(c)), \tag{A12} \]
where \( \text{spec}(A) \) is the spectrum of \( A \). Define
\[ S(\infty) := \lim_{c \to \infty} S(c) = \begin{bmatrix} 0 & 1 & 0 & 0 & \ldots & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & \ldots & 0 & 0 & 0 \\ 0 & \sigma_1 & 0 & 0 & \ldots & 0 & 0 & 0 \\ \vdots & & & & & & & \\ 0 & 0 & 0 & \ldots & a_{N-3} & 0 \end{bmatrix}. \tag{A13} \]

The characteristic polynomial of \( S(\infty) \) is \( \zeta := \det(zI_N - S(\infty)) = z^{N-2}(z - 1)(z + 1) \), so \( \sigma_1(S(\infty)) = 1 \) is a simple eigenvalue with corresponding eigenvector \( v(\infty) \), which is given by
\[ v_1(\infty) = v_2(\infty) = 1 \quad \text{and} \quad v_j(\infty) = \delta_{j-1,2} q_k \quad \text{for all } j \in \{3, \ldots, N\}. \tag{A14} \]

Using arguments similar to (A7) and taking the limit \( c \to \infty \) in (A11), and using the fact that \( \lim_{c \to \infty} \sigma(c)/c = 1 \) yields \( S(\infty)v(\infty) = v(\infty) \). By (A10), for any \( i = 1, \ldots, N - 2 \) we have
\[ \nu_i(\infty)/\nu_i(\infty) = c_{i-2}/c_{i+1} \neq 0. \]
This proves (A5).

In practice, \( c \) is positive (even if small), so it is useful to derive explicit error bounds in the asymptotic expressions. Note that since we are interested in ratios in entries of the Perron vector, we do not necessarily require it to be normalized. For two functions \( f, g: \mathbb{R}_+ \to \mathbb{R}_+ \), write \( f = O(g) \) if there exist \( c, y > 0 \) such that
\[ |f(x)| \leq c|g(x)| \quad \text{for any } x \geq y. \tag{A15} \]

The next result provides more explicit information on the Perron eigenvector of \( T(c, a) \).

**Theorem A.2.** Assume that the assumptions of theorem A.1 hold. Then as \( c \to \infty \), \( T(c, a) \) admits a Perron eigenvector \( \eta(c) \in \mathbb{R}_+^N \) satisfying
\[ \begin{aligned} \eta_1(c) & = 1 + O(c^{-2}), \\ \eta_2(c) & = 1 + O(c^{-2}) \\ & \vdots \\ \eta_i(c) & = a_1 \cdots a_{i-3} a_{i-2} c^{-2} + O(c^{-4}), \quad 3 \leq i \leq N. \end{aligned} \tag{A16} \]

In the proof of this result we actually provide explicit upper bounds for the asymptotic terms in (A16) (see equation (A29) below).

**Proof.** For a vector \( q \in \mathbb{R}_+^N \), let \( \text{sp}(q) := \{\eta|\eta \in \mathbb{R}\} \) denote the span of \( q \). Define an \( N \times N \) matrix \( R \) by
\[ R := c^2(S(c) - S(\infty)). \tag{A17} \]
Note that this implies that \( R \) does not depend on \( c \), and that
\[ \|R\| = \max_{1 \leq i \leq N} a_i. \tag{A18} \]
Let \( v(c) \) be the Perron eigenvector of \( S(c) \) defined in (A10), and let
\[ P(\infty) := \text{Proj}_{\text{sp}(v(\infty))} \quad \text{and} \quad P(c) := \text{Proj}_{\text{sp}(v(c))} \tag{A19} \]
be the projection operators on \( \text{sp}(v(\infty)) \) and \( \text{sp}(v(c)) \), respectively. Let \( \xi(c) := P(c)v(\infty) \). Since we project on \( \text{sp}(v(c)) \), this implies that \( \xi(c) \) is a Perron eigenvector of \( S(c) \) corresponding to \( \sigma(\infty)/c \). Let \( \gamma(c) := D^{-1}\xi(c) \). Then (A11) implies that \( \gamma(c) \) is a Perron eigenvector of \( T(c) \) corresponding to \( \sigma(c) \). We will show that \( \gamma(c) \) satisfies (A16).

We begin by analysing \( \xi(c) \). Let \( \Gamma \) be the circle in the complex plane parametrized by \( \gamma(t) = 1 + \frac{1}{2} \exp(it), t \in [0, 2\pi] \) (see figure 7).

**Proposition A.3.** For any \( c > 4||R|| \) we have that \( \sigma_1(S(c)) \) and \( \sigma_1(S(\infty)) = 1 \) are the only eigenvalues of \( S(c) \) and \( S(\infty) \), respectively, located in the interior of \( \Gamma \). All other (real) eigenvalues are to the left of the line \( \{s \in \mathbb{C}||R(s)| = 1/2\} \), where \( R(e) \) is the real part of \( s \).

**Proof.** Recall that \( \text{spec}(S(\infty)) = \{-1, 0, 1\} \). Hence, the claim is true for \( S(\infty) \). For \( S(c) \), consider first the corresponding \( T(c) \), satisfying (A9). Recall that all eigenvalues of \( T(c) \) are real. By theorem A.1 and (A18), we have
\[ \frac{\sigma_1(T(c))}{c} = 1 - \frac{2||R||}{c} + O\left(\frac{1}{c^2}\right) \subseteq \left(0, \frac{5}{4}\right). \]
Using Weyl's inequalities (A6), the second largest eigenvalue of \( T(c) \) satisfies \( \sigma_2(T(c)) \leq 2||R|| \). The proposition then follows from (A12).

It is well known [56, ch. 1] that the projections defined in (A19) satisfy the matrix representations
\[ P(\infty) = \frac{1}{2\pi} \int_{\Gamma} \left(A\lambda_N - S(\infty)\right)^{-1} \, d\lambda \tag{A20} \]
and
\[ P(c) = \frac{1}{2\pi} \int_{\Gamma} \left(A\lambda_N - S(c)\right)^{-1} \, d\lambda. \]
We require the following auxiliary result that provides a Neumann series representation for the difference between these two projections. The proof is provided for completeness. Let
\[ M_\Gamma := \max_{\lambda \in \Gamma} \|\lambda\lambda_N - S(\infty)\|^{-1}, \]

i.e. the maximal norm of the resolvent of \( S(\infty) \) over \( \Gamma \). This maximum exists since \( \lambda\lambda_N - S(\infty)\) is continuous in a neighbourhood of \( \Gamma \).

**Lemma A.4.** Fix \( \lambda \in \Gamma \). For any \( c > \max(\sqrt{M_\Gamma}||R||, 4||R||) \), we have
\[ (\lambda\lambda_N - S(c))^{-1} = (\lambda\lambda_N - S(\infty))^{-1} \sum_{k=0}^{\infty} (c^{-2}R(\lambda\lambda_N - S(\infty))^{-1})^k. \tag{A21} \]
In particular, the series on the right-hand side of (A21) converges absolutely and uniformly on \( \Gamma \), and
\[ P(c) = P(\infty) + \sum_{k=0}^{\infty} \frac{1}{2\pi} \int_{\Gamma} (\lambda\lambda_N - S(\infty))^{-1} (c^{-2}R(\lambda\lambda_N - S(\infty))^{-1})^k \, d\lambda. \tag{A22} \]
Figure 7. The curve $\Gamma$ in the complex plane. The eigenvalues of $S(\infty)$ are $-1$, $0$, $1$, implying that for any $c$ large enough all the eigenvalues of $S(c)$, except for $\sigma_1(S(c))$, are located outside of $\Gamma$. For presentation purposes, $\sigma_1(S(c))$ is assumed to be on the right of the point $[1,0]^t$.

**Proof.** Fix $\lambda \in \Gamma$. The definition of $R$ gives $(\lambda I_N - S(c))^{-1} = (\lambda I_N - S(\infty) - c^{-2}R)^{-1}$, so

\[
(\lambda I_N - S(c))^{-1} = [(\lambda I_N - S(\infty) - c^{-2}R)(\lambda I_N - S(\infty))^{-1}]^{-1} = (\lambda I_N - S(\infty))^{-1} - c^{-2}R(\lambda I_N - S(\infty))^{-1},
\]

and the series converges if $|c^{-2}R(\lambda I_N - S(\infty))^{-1}| < 1$. We have

\[
\|c^{-2}R(\lambda I_N - S(\infty))^{-1}\| \leq q(c) := c^{-2}M_R\|R\|,
\]

and $q(c) < 1$ for any $c > \max(\sqrt{M_R}\|R\|, 4\|R\|)$. Moreover, for any such $c$, we have

\[
\left\| (\lambda I_N - S(\infty))^{-1} \sum_{k=0}^{\infty} (c^{-2}R(\lambda I_N - S(\infty))^{-1})^k \right\| \leq M_R \sum_{k=0}^{\infty} (q(c))^k = M_R \frac{q(c)}{1 - q(c)} = O(c^{-2}),
\]

so the Neumann series converges absolutely and uniformly on $\Gamma$. This proves (A 21). Combining (A 20) and (A 21) proves (A 22), and this completes the proof of lemma A 4. \hfill \blacksquare

We can now prove theorem A 2. Define $\varepsilon(c) := (P(c) - P(\infty))\nu(\infty)$. Then

\[
\varepsilon(c) = P(c)\nu(\infty) - (P(c) - P(\infty) + P(\infty))\nu(\infty) = \varepsilon(c) + \nu(\infty),
\]

and using lemma A 4 gives

\[
|\varepsilon(c)| = \left| \left( \sum_{k=1}^{\infty} \frac{1}{2\pi} \int_{\Gamma} (\lambda I_N - S(\infty))^{-1} (c^{-2}R(\lambda I_N - S(\infty))^{-1})^k \, d\lambda \right) \nu(\infty) \right| \leq \frac{M_R}{2} \frac{q(c)}{1 - q(c)} |\nu(\infty)| = O(c^{-2}).
\]

Now, $\eta(c) = D^{-1}\varepsilon(c)$ gives

\[
\eta(c) - D^{-1}\nu(\infty) = D^{-1}\varepsilon(c).
\]

We conclude that for any $c > \max(\sqrt{M_R}\|R\|, 4\|R\|)$, we have

\[
|\eta(c)| \leq \left| \sum_{k=1}^{\infty} \frac{1}{2\pi} \int_{\Gamma} (\lambda I_N - S(\infty))^{-1} (c^{-2}R(\lambda I_N - S(\infty))^{-1})^k \, d\lambda \right| \nu(\infty) \leq \frac{M_R}{2} \frac{q(c)}{1 - q(c)} |\nu(\infty)| \leq \frac{M_R}{2} \frac{q(c)}{1 - q(c)} |\nu(\infty)|.
\]

Note that given $c > \max(\sqrt{M_R}\|R\|, 4\|R\|)$, the upper bounds in (A 27), (A 28) and (A 29) can be computed explicitly using the expressions for the vector $\nu(\infty)$ in (A 14), the norm $\|R\|$ in (A 18), and the expression for $q_i$ in (A 23). Combining these upper bounds with (A 24) yields (A 16), and this completes the proof of theorem A 2. \hfill \blacksquare

**Remark A 5.** Note that approximations of the coordinates of $\eta(c)$ up to arbitrarily small error can be obtained by computing higher order terms in the Neumann series (A 22). As an example, consider for simplicity the case $a_i = 1$ for $i = 1, \ldots, N - 2$, which implies $\|R\| = 1$. Assume that $c > \max(\sqrt{M_R}, 4)$ and let $\delta > 0$ be the desired error bound. Let $L > 0$ be a sufficiently large integer such that

\[
\frac{(q(c))^L}{1 - q(c)} < \frac{2\delta}{|\nu(\infty)|M_R}.
\]

Then, arguments similar to (A 24) imply that

\[
\left\| (\lambda I_N - S(\infty))^{-1} \sum_{k=1}^{\infty} (c^{-2}R(\lambda I_N - S(\infty))^{-1})^k \, d\lambda \right\| \nu(\infty) \leq \frac{M_R(q(c))^L}{1 - q(c)}.
\]

Hence, $\varepsilon(c) = \mu(c) + \tilde{\varepsilon}(c)$, where

\[
|\tilde{\varepsilon}(c)| = \left| \sum_{k=1}^{\infty} \frac{1}{2\pi} \int_{\Gamma} (\lambda I_N - S(\infty))^{-1} (c^{-2}R(\lambda I_N - S(\infty))^{-1})^k \, d\lambda \right| \nu(\infty) \leq \frac{M_R(q(c))^L}{2} \frac{q(c)}{1 - q(c)} |\nu(\infty)| < \delta.
\]

\[
\sigma_j(S(c)), j \geq 2
\]

\[
\sigma_{\max}(S(c))
\]

\[
1/2
\]

\[
\mathbb{R}
\]
The vector
\[
\mu(c) = \left( \sum \frac{1}{m_j} \right) \int_{\Lambda} \left( \lambda I_N - S(\infty) \right)^{-1} (c - R(\lambda I_N - S(\infty))^{-1})^j \, d\lambda \left( \nu(\infty) \right)
\]
(A31)

can be computed explicitly by the Cauchy residue theorem \cite{37}. Using the fact that \(s = D - \xi(c)\) yields
\[
\max_{1 \leq i \leq N} |\eta(c) - \frac{1}{d_i} \mu(c)| = \frac{1}{d_i} |\eta_i(c)| \leq \frac{1}{d_i} |\xi(c)| < \delta,
\]
so the entries of \(D^{-1}\mu(c)\) approximate those of \(\eta(c)\) with an error smaller than \(\delta\).

The next result provides an explicit expression for \((\lambda I_N - S(\infty))^{-1}\) with \(\lambda \in \Gamma\). This can be used to derive an upper bound on the constant \(M_1\), given in (A 23), thereby leading to an explicit estimate of the errors in (A 29). Moreover, \((\lambda I_N - S(\infty))^{-1}\) can be substituted into (A 31) to obtain high-order approximations of the Perron eigenvector.

**Proposition A.6.** Let \(\Gamma\) be as in figure 7. Then, for any \(\lambda \in \Gamma\)
\[
(\lambda I_N - S(\infty))^{-1} = \begin{bmatrix} D & 0_{2s(N-2)} \\ EBD & \frac{0_{2s(N-2)}}{E} \end{bmatrix},
\]
where
\[
D = \frac{1}{\lambda^2 - 1} \begin{bmatrix} \lambda & 1 \\ 1 & \lambda \end{bmatrix}, \quad E = \begin{bmatrix} 0, & \vdots, & 0 \end{bmatrix} \quad \text{and} \quad B = \begin{bmatrix} 0 & -\alpha_1 & \vdots & 0 \\ \vdots & \ddots & \ddots & \vdots \\ 0 & \cdots & 0 & -\alpha_{N-1} \end{bmatrix}.
\]

Proof. We can divide \(\lambda I_N - S(\infty)\) into blocks as
\[
\lambda I_N - S(\infty) = \begin{bmatrix} A & 0_{2s(N-2)} \\ B & C \end{bmatrix},
\]
with
\[
A := \begin{bmatrix} \lambda & -1 \\ -1 & \lambda \end{bmatrix} \quad \text{and} \quad C := \begin{bmatrix} \lambda & 0 & 0 & \cdots & 0 & 0 \\ 0 & \lambda & 0 & \cdots & 0 & 0 \\ \vdots & \ddots & \ddots & \ddots & \vdots & \vdots \\ 0 & \cdots & 0 & \lambda & -\alpha_2 \\ 0 & \cdots & 0 & -\alpha_2 & \cdots & \cdots \\ \vdots & \cdots & \cdots & \cdots & \cdots & \cdots \end{bmatrix}.
\]

and \(B\) defined in (A 33). Since \(\lambda \neq 0\), it can be easily verified that \(C^{-1} = E\) and \(A^{-1} = D\). Hence,
\[
\begin{bmatrix} A & 0_{2s(N-2)} \\ B & EBD \end{bmatrix} \begin{bmatrix} D & 0_{2s(N-2)} \\ \frac{0_{2s(N-2)}}{E} \end{bmatrix} = \begin{bmatrix} AD & 0_{2s(N-2)} \\ BD - CEBD & CE \end{bmatrix} = I_N,
\]
and this completes the proof.

We can now prove the results in §4.

**A.1. Proof of proposition 4.1.**
It was shown in \cite{34} that for any \(s > 0\) the network satisfies the following persistence property for any initial condition. After an arbitrarily short time the pool density is positive, and all the densities in the sites are larger than zero and smaller than one. In particular, this implies that
\[
\epsilon_s(q) > 0 \quad \text{and} \quad 0 < \epsilon_s^p(q) < 1
\]
(A34)

for any integer \(q > 0\), any \(p \in \{1, \ldots, q\}\), and any \(j \in \{1, \ldots, n_p\}\).

Seeking a contradiction, assume that the claim in proposition 4.1 is not true. Then there exists an integer \(m > 0\) such that
\[
\epsilon_s(m + 1) \geq \epsilon_s(m) > 0.
\]
(35)

Let
\[
S(q, m) = \sum_{j=1}^{n_p} \rho_j(q),
\]
i.e. the total steady-state density of ribosomes in RFMIO \(\#i\) when the network includes \(q\) RFMIOs. By (A 34), we have \(S(m + 1, m + 1) > 0\). Taking into account (A 35) and the fact that the total number of ribosomes is fixed at \(s\), we conclude that there exists some \(1 \leq p \leq m\) such that
\[
S(p, m + 1) < S(p, m),
\]
i.e. the total steady-state density of ribosomes along RFMIO \(\#p\) has decreased due to the addition of RFMIO \(\#(m + 1)\) to the network. In particular, for at least one site \(j \in \{1, \ldots, n_p\}\), we have
\[
\epsilon_s^p(m + 1) < \epsilon_s^p(m).
\]
(36)

Consider the steady-state equations of RFMIO \(\#p\) when the network has \(q\) RFMIOs, namely,
\[
\begin{align*}
\text{Site 1:} & \quad G_p(\epsilon_s(q)) \lambda_1^p(1 - \epsilon_s^p(q)) - \lambda_2^p \epsilon_s^p(q)(1 - \epsilon_s^p(q)), \\
\text{Site 2:} & \quad \lambda_3^p \epsilon_s^p(q)(1 - \epsilon_s^p(q)) - \lambda_4^p \epsilon_s^p(q)(1 - \epsilon_s^p(q)), \\
\text{Site 3:} & \quad \lambda_5^p \epsilon_s^p(q)(1 - \epsilon_s^p(q)) - \lambda_6^p \epsilon_s^p(q)(1 - \epsilon_s^p(q)), \\
& \vdots \\
\text{Site } n_p - 1: & \quad \lambda_{n_p-2}^p \epsilon_s^p(q)(1 - \epsilon_s^p(q)) - \lambda_{n_p-1}^p \epsilon_s^p(q)(1 - \epsilon_s^p(q)), \\
\text{Site } n_p: & \quad \lambda_{n_p}^p \epsilon_s^p(q)(1 - \epsilon_s^p(q)) - \lambda_{n_p+1}^p \epsilon_s^p(q).
\end{align*}
\]
(A37)
Define a function $\psi(0, 1)^2 \to \mathbb{R}$ by $\psi(x, y) := x/(1 - y)$, and note that
\[ x_1 \leq x_2 \quad \text{and} \quad y_1 \leq y_2 \Rightarrow \psi(x_1, y_1) \leq \psi(x_2, y_2). \quad (A38) \]
eqation (A37) with $q = m + 1$ yields
\[
eqation (A39) \]

All these expressions are well-defined by (A34). We now consider two cases.

Case 1. Suppose that
\[ e_{i_1}(m + 1) \geq e_{i_1}(m), \]
i.e. the density in the last site of RFMIO $\#p$ did not decrease due to the addition of RFMIO $\#(m + 1)$. By backward induction in (A39) and using (A38), we immediately obtain that
\[ e_{i_1}(m + 1) \geq e_{i_1}(m) \quad \text{for all } j = 1, \ldots, n_p. \]
This contradicts (A36).

Case 2. Suppose that
\[ e_{i_1}(m + 1) < e_{i_1}(m), \quad (A40) \]
i.e. the density in the last site of RFMIO $\#p$ decreased due to the addition of RFMIO $\#(m + 1)$. By backward induction and (A38), we find that
\[ e_{i_1}(m + 1) < e_{i_1}(m) \quad \text{for all } j = 1, \ldots, n_p. \quad (A41) \]
Now using (A37) with $q = m$ and $q = m + 1$ gives
\[
\frac{e_{i_1}(m + 1)}{e_{i_1}(m)} = \frac{G_p(e_{i_1}(m + 1))}{G_p(e_{i_1}(m))} \frac{1 - e_{i_1}(m + 1)}{1 - e_{i_1}(m)},
\]
and using (A35), the monotonicity of $G_p$, and (A41) yields ($e_{i_1}(m + 1)/e_{i_1}(m)) > 1$. This contradicts (A40).

Summarizing, we see that (A35) cannot hold, and this completes the proof.

A.2. Proof of proposition 4.3
Seeking a contradiction, assume that the claim is not true. Then there exists $\beta > 0$ and a sequence $m_1 < m_2 < \ldots$ such that $e_s(m_k) \geq \beta$ for all $k$. For any $k$ and any $i = 1, \ldots, m_k$, let
\[ S(i, m_k) := \sum_{j=1}^{m_k} e_i(m_k), \]
i.e. the total density of ribosomes along RFMIO $\#i$ at steady state when the network includes $m_k$ RFMIOs. Since the total number of ribosomes in the network is $s$ (and this is independent of $m_k$), we have
\[
S = n_i(m_k) + \sum_{i=1}^{m_k} S(i, m_k), \quad (A42) \]
Note that the bound on the right-hand side of (A42) does not depend on $m_k$. Let
\[
\varepsilon := \min \left\{ \frac{\^p_m \lambda_s}{4\lambda - 2} \right\}. \quad (A43) \]
Consider the network with $m_k$ RFMIOs initialized at the equilibrium $e(m_k)$ at time $t = 0$. Then for any time $t \geq 0$, we have
\[
0 \leq \sum_{i=1}^{m_k} S(i, m_k) \leq s - \beta - \varepsilon. \quad (A42) \]
Note that $\varepsilon \in (0, 1/2]$. If for any $m_k$ and any $i \in \{1, \ldots, m_k\}$ we have that $S(i, m_k) > \varepsilon$ then for a large enough $k$ this contradicts (A42). Therefore, there exist $k$ and $p \in \{1, \ldots, m_k\}$ such that $S(p, m_k) \leq \varepsilon$. In particular,
\[
eqation (A43) \]
Note that $\varepsilon$ is independent of $m_k$. Let
\[
\varepsilon := \min \left\{ \frac{\^p_m \lambda_s}{4\lambda - 2} \right\}. \quad (A43) \]
Consider the network with $m_k$ RFMIOs initialized at the equilibrium $e(m_k)$ at time $t = 0$. Then for any time $t \geq 0$, we have
\[
\sum_{i=1}^{m_k} \dot{e}_i(m_k) \geq \sum_{i=1}^{m_k} G_p(e_{i}(m_k)) \lambda_s^p (1 - e_i^p) - \lambda_s^p e_i^p \]
\[
\geq G_p(\beta) \lambda_s^p (1 - e_i^p) - \lambda_s^p e_i^p \]
\[
\geq g, \beta \lambda_s^p \frac{1}{2} - \lambda_s^p e_i^p \]
\[
> 0, \]
where the second line follows from the monotonicity of the pool output functions, the third from the assumptions in the
statement of the proposition and (A 43), and the fourth from the definition of $\varepsilon$. However, since the system is initialized at the steady state, $\sum_{j=1}^{m} c_j^i = 0$ for all $i \geq 0$. This contradiction completes the proof.

A.3. Proof of proposition 4.4

Fix $i \in \{1, \ldots, m\}$, and consider RFMIO #i. Setting $N = n_i + 2$, $c = (\lambda^i_j G_j(\varepsilon_j))^{-(1/2)}$, and $\alpha_j = (\lambda^i_j)^{-(1/2)}$, $j = 1, \ldots, n_i$, (A 44)

in (A 1), we have that $T_c[\varepsilon, \alpha]$ is the matrix whose Perron eigenvalue $\sigma$ and eigenvector $\mathbf{c}$ are used to compute the steady state in RFMIO #i via (2.4), that is,

$$
c^i_j = \frac{c^i_j}{\lambda^i_j}, \quad j = 1, \ldots, n_i.
$$

When $\varepsilon_c \to 0$, $c \to \infty$, and applying (A 3) and (A 5) yields (4.4).

If the pool output function $G_i$ is differentiable at zero, then

$$
\lim_{\varepsilon \to 0^+} c^i_j = \lim_{\varepsilon \to 0^+} \left[ \frac{c^i_j (G_j(\varepsilon_j) - G_j(0))}{\varepsilon_j} \right],
$$

where we used the fact that $G_j(0) = 0$. Equation (4.5) now follows from (4.4). This completes the proof of proposition 4.4.

A.4. Proof of corollary 4.6

Assume a sequence of networks with $m_p$ RFMIOs, $p = 1, 2, \ldots$, with $\lim_{p \to \infty} m_p = \infty$. For any $p$, let $\varepsilon_p(p)$ be the corresponding steady-state pool density. By proposition 4.3, $\lim_{p \to \infty} m_p = \infty$. For any $p$, let $f_j(p)$ be the ratio of the total production rate and the pool density for the case of $m_p$ RFMIOs in the network. By proposition 4.4, the functions $\{f_j(p)\}$ converge pointwise to $f(x) = \sum_{i=1}^{m} \lambda^i_j G_j(0)$. By Fatou’s lemma [58, Theorem 2.8.3]

$$
\liminf_{p \to \infty} TPR(\varepsilon_j) = \liminf_{p \to \infty} \int_{-\infty}^{\infty} f_j(p(x)) dx \geq \int_{-\infty}^{\infty} f(x) dx = \sum_{i=1}^{m} \lambda^i_j G_j(0). \tag{4.7}
$$

The last equality follows from assumption 4.2, which implies that $\lambda^i_j G_j(0) \geq \lambda^i_j > 0$. In the case where the number of RFMIOs is fixed at $m$ as $\varepsilon \to 0$, by replacing Fatou’s lemma with the dominated convergence theorem [58, Theorem 2.8.1], the limit-inferior can be replaced by a limit, and the inequality can be replaced by equality. Note that in this case $\sum_{i=1}^{m} \lambda^i_j G_j(0) < \infty$. This proves (4.8).

To prove (4.9), consider first the case where the number of RFMIOs $m$ is fixed and the total density in the network satisfies $s \to 0$. Recall that $q = F(\varepsilon)/s$, and (4.7) gives $q = (1 + \sum_{i=1}^{m} \lambda^i_j G_j(0)^{-1})^{-1}$. Using (4.5) yields

$$
\lim_{\varepsilon \to 0^-} q = \lim_{m \to \infty} \left[ 1 + \sum_{i=1}^{m} \lambda^i_j G_j(0)^{-1} \right]^{-1}
$$

$$
= \left[ 1 + \sum_{i=1}^{m} \lambda^i_j G_j(0)^{-1} \right]^{-1}
$$

and this completes the proof. We note in passing that in the case where the total ribosome density in the network $s$ is fixed and the number of RFMIOs satisfies $m \to \infty$, assumption 4.2 gives

$$
\frac{\lambda^i_j G_j(0)}{\lambda^i_j} \geq \frac{\lambda^i_j}{\lambda^i_j} \quad \text{for all } i,
$$

so in this case we have

$$
\lim_{\varepsilon \to 0^-} q = \lim_{m \to \infty} \left[ 1 + \sum_{i=1}^{m} \lambda^i_j G_j(0)^{-1} \right]^{-1} = 0.
$$

A.5. Proof of proposition 4.8

Using (4.8) gives $\lim_{\varepsilon \to 0} TPR(\varepsilon) = \lambda_{\text{avg}}$, and combining this with (4.10) gives (4.12). Similarly, equation (4.9) gives

$$
\lim_{\varepsilon \to 0} \frac{g(\alpha\varepsilon)}{H(\lambda_1, \ldots, \lambda_m)} = \lim_{\varepsilon \to 0} \frac{g(\alpha\varepsilon)}{H(\lambda_1, \ldots, \lambda_m)} = 0.
$$

and using (4.10) and (4.11) implies that these expressions are equal. This completes the proof of proposition 4.8.

References

1. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. 2008 Molecular biology of the cell. New York, NY: Garland Science.
2. Haussler J, Mayo A, Keren L, Alon U. 2019 Central dogma rates and the trade-off between precision and economy in gene expression. Nat. Commun. 10, 1–15. (doi:10.1038/s41467-018-07391-8)
3. Tuller T, Vekler I, Gazit N, Kupiec M, Ruppin E, Ziv M. 2011 Composite effects of gene determinants on the translation speed and density of ribosomes. Genome Biol. 12, R110. (doi:10.1186/gb-2011-12-11-r110)
4. Diament A, Feldman A, Schochet E, Kupiec M, Aarav Y, Tuller T. 2018 The extent of ribosome queuing in budding yeast. PLoS Comput. Biol. 14, 1–21. (doi:10.1371/journal.pcbi.1005951)
5. Han P et al. 2020 Genome-wide survey of ribosome collision. Cell Rep. 31, 107610. (doi:10.1016/j.celrep.2020.107610)
6. Sauna ZE, Kimchi-Sarfaty C. 2011 Understanding the contribution of synonymous mutations to human disease. Nat. Rev. Genet. 12, 683–691. (doi:10.1038/nrg3051)
7. Subramanian S. 2021 Ribosome traffic jam in neurodegeneration: decoding hurdles in Huntington disease. Cell Stress 5, 86–88. (doi:10.15698/cst2021.06.251)
8. Ingolia NT. 2014 Ribosome profiling: new views of translation, from single codons to genome scale. Nat. Rev. Genet. 15, 205–213. (doi:10.1038/nrg3645)
9. Insbergh MV, van den Berg J, Andersson-Rolf A, Clevers H, van Oudenaarden A. 2021 Single-cell Rib-seq reveals cell cycle-dependent translational pausing. Nature 597, 561–565. (doi:10.1038/s41586-021-03887-4)
10. Zlotorynski E. 2016 Live stream: translation at single-mRNA resolution. Nat. Rev. Mol. Cell Biol. 17, 395. (doi:10.1038/nrm.2016.78)
11. Finkel Y et al. 2021 SARS-CoV-2 uses a multipronged strategy to impede host protein
