On the gain of entrainment in the $n$-dimensional ribosome flow model

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The ribosome flow model (RFM) is a phenomenological model for the flow of particles along a one-dimensional chain of $n$ sites. It has been extensively used to study ribosome flow along the mRNA molecule during translation. When the transition rates along the chain are time-varying and jointly $T$-periodic the RFM entrains, i.e. every trajectory of the RFM converges to a unique $T$-periodic solution that depends on the transition rates, but not on the initial condition. In general, entrainment to periodic excitations like the 24 h solar day or the 50 Hz frequency of the electric grid is important in numerous natural and artificial systems. An interesting question, called the gain of entrainment (GOE) in the RFM, is whether proper coordination of the periodic translation rates along the mRNA can lead to a larger average protein production rate. Analysing the GOE in the RFM is non-trivial and partial results exist only for the RFM with dimensions $n = 1, 2$. We use a new approach to derive several results on the GOE in the general $n$-dimensional RFM. Perhaps surprisingly, we rigorously characterize several cases where there is no GOE, so to maximize the average production rate in these cases, the best choice is to use constant transition rates all along the chain.

1. Introduction

The totally asymmetric simple exclusion process (TASEP) is a fundamental model in statistical mechanics [1–3]. The basic version of TASEP includes $n$ sites, ordered along a one-dimensional lattice, and particles that hop randomly in a unidirectional manner, i.e. from site $i$ to site $i+1$. Each site can be either empty or include a particle, and a particle can only hop into an empty site. This generates an intricate coupling between the particles. In particular, if a particle is ‘stuck’ in a site for a long time then particles will accumulate in the sites behind it, thus generating a ‘traffic jam’ of particles.

TASEP has been used to model the flow of ribosomes along mRNA [4] and other processes of intracellular transport [5], pedestrian and vehicular traffic [6], the movement of ants, and numerous other natural and artificial phenomena [3]. However, rigorous analysis of TASEP is difficult and closed-form results are known only for very special cases of transition rates along the lattice [1].

The ribosome flow model (RFM) is the mean-field approximation of TASEP [7]. The RFM is a deterministic model composed of $n$ nonlinear, first-order ODEs. The RFM is highly amenable to analysis using tools from systems and control theory: the RFM is a contracting system [8], a cooperative system [9], and, moreover, it is also a totally positive differential system [10]. The RFM can also be interpreted as a compartmental chemical reaction network with transition rates that depend on the amount of particles and free space in various compartments [11]. The RFM can also be derived via a special finite volume spatial discretization of widely used hyperbolic PDE flow models [12], and can also be represented as a port-Hamiltonian system [13].
Understanding the regulation and dynamics of ribosome flow and, consequently, protein production rate is a fundamental problem in biology and medicine. For example, ‘traffic jams’ of ribosomes along mRNA have been implicated with various diseases [14,15]. Rigorous analysis of ribosome flow is also important in scientific fields that include manipulations of the translation machinery like synthetic biology, mRNA viruses and biotechnology.

The RFM and its variants have been extensively used to model and analyse the flow of ribosomes along mRNA during translation (e.g. [16–26]). More recently, networks of interconnected RFMs have been used to model and analyse large-scale translation in the cell, and the effect of competition for shared resources like ribosomes and tRNA molecules [27–31]. Models based on networks of RFMs have also been validated experimentally. It was shown that such a model can predict the density of ribosomes along different mRNAs, the protein levels of different genes, and can even be used for engineering ribosomal traffic jams (e.g. [7,32,33]).

Margaliot et al. [34] studied the RFM when the entry, exit, and elongation rates along the chain are jointly periodic, with a common period $T$, and proved that in this case, the dynamics admits a unique $T$-periodic solution that is globally exponentially stable (GES). If we view the rates as a periodic excitation (e.g. due to the periodic cell-cycle process) then the state variables in the RFM, and thus also the protein production rate, entrain to the excitation.

The cell cycle is a periodic programme that controls DNA synthesis and cell division. Proper execution of the cell-cycle programme, and its coordination with cell growth, entails the expression and activation of key proteins at specific times along the period [35]. The eukaryotic cell cycle is controlled by periodic gene expression [36]. There is growing evidence that protein levels of cell-cycle-related genes are regulated also via the translation machinery. Higanea-Mendoza & Pardo-Galvan [37] report that the expression of the human translation initiation factor 3 (eIF3) oscillates during cell cycle, with one maximum expression peak in the early S phase and a second during mitosis. Frenkel-Morgenstern et al. [38] argue that periodicity in the tRNA levels of bottleneck codons induces periodicity in the translation rate. Patil et al. [39] showed that the levels of 16 tRNA modifications, and thus the translation efficiencies of different codons oscillate during cell cycle. Their results imply that translation regulation has a direct role in cell-cycle-related oscillations. Another possible regulation mechanism is via control of the transcription rate of tRNA genes (and other genes), resulting in oscillations in intra-cellular tRNA levels [12]. Since the decoding time of codons is affected by the available levels of the tRNA molecules recognizing them, this may eventually lead to oscillations in the decoding times of different codons. A recent paper [40] used a sophisticated single-cell ribo-seq method to show that limitation for a particular amino acid causes ribosome pausing at a subset of the codons encoding the amino acid. However, this pausing is only observed in a sub-population of cells correlating to its cell cycle state.

Other studies [41,42] considered the gain of entrainment (GOE) in the RFM. To explain the GOE problem, consider a general nonlinear control system

\[
\begin{align*}
\dot{x}(t) &= f(x(t), u(t)) \\
y(t) &= g(x(t)),
\end{align*}
\]

where $x \in \mathbb{R}^n$ is the state vector, $u \in \mathbb{R}^m$ is the input, and $y \in \mathbb{R}^l$ is the scalar output. We assume that the output represents a quantity that should be maximized (in some suitable sense). For example, in the context of biotechnology, the output may be the production rate of a desired protein.

A control $u$ is called $T$-periodic if $u(t+T) = u(t)$ for all $t \geq 0$. Suppose that for any $T$-periodic control the system (1.1) entrains, that is, $x$ converges to a unique $T$-periodic solution $\gamma(t)$, $t \in [0, T)$, that depends on the control, but not on the initial condition $x(0)$. Then the output converges to the scalar $g(\gamma)$. Now fix a $T$-periodic control $u$, and let

\[
\pi := \frac{1}{T} \int_0^T u(t) \, dt
\]

and

\[
y := \frac{1}{T} \int_0^T g(\gamma(t)) \, dt,
\]

i.e. the averages of the input and the unique periodic output. If we apply the constant control $v(t) := \pi$ then the entrainment property implies that the state will converge to a unique equilibrium $\epsilon$, that depends on $\pi$, but not on the initial condition $x(0)$, and thus $y(t)$ converges to $g(\epsilon)$. We say that the system admits a GOE for the input $u$ if

\[
y > g(\epsilon).
\]

In other words, we consider two controls $u(t)$ and $v(t) := \pi$ with the same average, and compare the average of the corresponding asymptotic outputs. A GOE implies that entrainment does not only entail synchronization to periodic excitations, but also an improvement in the average output. This general property may be relevant in many fields including protein production during the cell cycle, division programme, seasonal cycles of infectious diseases [43], periodic fishery [44] and periodically operated chemical reactors [45].

Since periodic controls allow more flexibility than constant controls, one may intuitively expect that many systems admit GOE. However, analysing the GOE in a nonlinear system is non-trivial. Many nonlinear systems do not entrain at all, that is, the response to a periodic input is not necessarily convergence to a periodic solution (e.g. [46]). Even if the system does entrain, the periodic solution $\gamma(t)$ (and its integral along a period) is typically not known explicitly.

A dynamical system is called contractive if any two solutions approach each other at an exponential rate [8,47]. Contractive systems entrain to periodic excitations [8,48] and also admit a well-defined frequency response function [49], but again this is not known explicitly. Thus, rigorous analysis of the GOE is not immediate even for scalar contractive systems. The next example demonstrates this in a simple synthetic system.

**Example 1.1.** Consider the scalar control system

\[
x(t) = 1 - \frac{3}{2} \tanh(x(t)) + u(t)
\]

and

\[
y(t) = x(t).
\]

Note that this is in the form in (1.1) with $f(x, u) = 1 - \frac{3}{2} \tanh(x) + u$ and $g(x) = x$. Assume that $u(t)$ takes values
in \([-1, 1]\). The state space of the dynamics is \(\Omega := \mathbb{R}_+\). The Jacobian of the vector field is \(f(x) = -3/(2\cosh^2(x))\), so in particular \(f(x) < 0\) for all \(x \in \Omega\) implying that the system is contractive and thus entrains to periodic excitations. We consider two controls. The first is \(u(t) = \sin(2\pi t)\) that is \(T\)-periodic, with \(T = 1\), and has average \(\bar{u} = 0\), and the second is the constant control \(\bar{u}(t) \equiv 0\). For the latter control, the solution converges to the equilibrium point \(c = \tanh^{-1}(2/3) \approx 0.8047\), so the output converges to \(g(\bar{c}) = c\). Figure 1 depicts the attracting periodic solution \(\gamma(t)\), \(t \in [0, 1]\), for the control \(u(t)\). Numerically computing the average of this solution over a period yields \(\bar{\gamma} = \int_0^1 \gamma(t) \, dt = 0.8127\), and thus the system admits GOE for this control.

Analysing GOE is closely related to periodic optimal control [50]. The basic idea is to pose the problem of maximizing the average of the output, along the periodic solution, under an integral constraint on the control, namely, 

\[
\frac{1}{T} \int_0^T u(t) \, dt = \bar{u}.
\]

However, it seems that the application of standard analysis tools like Pontryagin’s maximum principle to this problem provides explicit information only for low-dimensional systems, e.g. when \(n = 1\) [51,52]. Another known approach for proving the optimality of equilibrium solutions based is on passivity/dissipativity techniques [53]. However, so far for the RFM we have not been able to establish the kind of passivity/dissipativity that we would need for this kind of analysis. While dissipativity results for generalized RFMs exist [13], it is not clear how they can be extended such that the transition rates play the role of the control, which would be needed for a GOE analysis with respect to periodic variations of these rates. Moreover, the integral constraints on the control cause technical difficulties.

Protein synthesis is known to be one of the most energy consuming processes in the cell [54]. A GOE may make this process more efficient on average, and thus reduce the effective cost. To study this problem in the context of translation, we develop a new approach to analysing GOE in the \(n\)-dimensional RFM. This is based on integrating the differential equations and ‘higher-order moments’ of these differential equations along the periodic solution.

It turns out that the algebraic derivations are simplified if we actually consider the differential equations for the centred variables obtained by subtracting from each state variable its equilibrium point corresponding to the case of constant controls \(u_i(t) = \bar{u}_i\) for all \(i\). Using this, we prove several new results (see theorem 3.1 below). For example, we show that for an \(n\)-dimensional RFM, with \(n\) odd, and equal internal rates there is no GOE. To the best of our knowledge, these are the first analysis results on the GOE in a general \(n\)-dimensional RFM.

The remainder of this paper is organized as follows. The next section reviews the RFM and formally defines the GOE in the RFM. Section 3 presents our main results. These show that in various \(n\)-dimensional RFMs there is no GOE. The final section concludes the paper.

### 2. Preliminaries

In this section, we briefly review the RFM, and the problem of analysing GOE.

#### 2.1. The ribosome flow model

The RFM is a phenomenological compartmental model that includes \(n\) sites ordered along a one-dimensional chain. Particles flow along the chain from left to right (i.e. from site 1 to site \(i + 1\)). The RFM includes \(n\) state variables \(x_i(t), i = 1, \ldots, n\). Every state variable takes values in \([0, 1]\), where \(x_i(t)\) represents the normalized density of particles at site \(i\) at time \(t\).

Thus, \(x_i(t) = 0\) \([x_i(t) = 1]\) implies that site \(i\) is empty [completely full] at time \(t\). The state-space of the RFM is thus the \(n\)-dimensional unit cube \([0, 1]^n\).

The RFM also includes \(n\) positive time-varying transition rates, \(\omega_i(t), \ldots, \omega_n(t)\), where a large value of \(\omega_i(t)\) implies that it is relatively easy for a particle to move from site \(i\) to site \(i + 1\) at time \(t\). In particular, \(\omega_i(t) (\omega_i(t))\) is called the initiation rate [exit rate] at time \(t\).

In the context of translation, every site corresponds to a group of codons along mRNA, and \(\omega_i(t)\) models biophysical properties like the abundance of cognate tRNA molecules at time \(t\).

The RFM consists of \(n\) deterministic first-order ODEs

\[
\begin{align*}
\dot{x}_i(t) &= \omega_{i-1}(t)x_{i-1}(t)(1 - x_i(t)) - \omega_i(t)x_i(t)(1 - x_{i+1}(t)), \\
&= \omega_{i-1}(t)x_{i-1}(t) - \omega_i(t)x_i(t) + \omega_i(t)x_{i+1}(t) \\
&= \omega_0(t)x_0(t) - \omega_1(t)x_1(t) + \omega_1(t)x_1(t) \\
\end{align*}
\]

where \(x_0(t) := 1\) and \(x_{n+1}(t) := 0\). If we view the transition rates as controls, then this is a nonlinear control system, as the equations include products of the state variables and the controls.

For example, the RFM with \(n = 3\) is given by

\[
\begin{align*}
\dot{x}_1 &= \omega_0(1 - x_1) - \omega_1(x_1 - x_2), \\
\dot{x}_2 &= \omega_1(x_1 - x_2) - \omega_2(x_2 - x_3), \\
\dot{x}_3 &= \omega_2(x_2 - x_3) - \omega_3(x_3) \\
\end{align*}
\]

where for the sake of simplicity we omit specifying the dependence on \(t\).

To explain these equations, consider the equation for \(x_2\) in (2.2). The term \(\omega_1(x_1 - x_2)\) is the flow from site 1 to site 2. This is proportional to: the transition rate \(\omega_1\) from site 1 to site 2; the density \(x_1\) of particles in site 1; and the ‘free space’ \(1 - x_2\) in site 2. Similarly, the term \(\omega_2(x_2 - x_3)\) is the flow from site 2 to site 3. Thus, the equation for \(x_2\) states that the change in density in site 2 is the flow into site 2 minus the flow out of site 2.

The output rate from site \(n\) is \(R(t) := \omega_n(t)x_n(t)\). When modelling translation, this corresponds to ribosomes exiting mRNA, and thus to the protein production rate. In the
context of biotechnology, a natural goal is to maximize this production rate. More generally, in many transportation systems modelled using the RFM a natural goal is to maximize \( R(t) \) (in some well-defined sense).

Just like TASEP, the RFM allows one to model and analyse the evolution of traffic jams of particles. This is due to the fact that the entry rate to site \( i \) depends on the free space \( 1 - x_i \), which may be interpreted as a ‘soft version’ of the simple exclusion principle. To explain this, assume that \( u_i(t) = \varepsilon \), with \( \varepsilon \) positive and close to zero. Then

\[
\dot{x}_2 \approx u_1 x_1 (1 - x_2) \geq 0,
\]

so we expect \( x_2 \) to increase towards one. Then \( 1 - x_2 \) decreases towards zero, so

\[
x_1 \approx u_0 (1 - x_1) \geq 0,
\]

so \( x_1 \) will also increase towards one. In this way, a traffic jam of particles is formed behind the slow transition rate.

Consider positive and jointly \( T \)-periodic transition rates \( u_0(t), \ldots, u_n(t) \), and denote the average of \( u_i(t) \) along a period by \( \bar{u}_i \). Margaliot et al. [34] showed that in this case the RFM admits a unique \( \gamma \)-periodic solution \( \gamma \), satisfying \( \gamma(t) \in (0, 1)^n \) for all \( t \in [0, T) \). The next example demonstrates this.

**Example 2.1.** Consider an RFM with dimension \( n = 3 \), positive and \( 2\pi \)-periodic rates \( u_0(t) = 3 + \cos(t + 5) \), \( u_1(t) = 1 \), \( u_2(t) = 4 + 2\sin(t - 4) \), \( u_3(t) = -2 - \cos(t - 1) \). Figure 2 depicts the solutions \( x_i(t) \) for the (arbitrarily chosen) initial condition \( x(0) = [0.3 0.4 0.5] \). It may be seen that every \( x_i(t) \) converges to a \( 2\pi \)-periodic solution \( \gamma \). The convergence to this \( \gamma \) holds for any initial condition \( x(0) \in [0, 1]^3 \).

In particular, if the transition rates are set to constant values \( u_i(t) = \bar{u}_i \) for all \( i \) then the RFM admits a unique \( \gamma \)-periodic equilibrium point \( \varepsilon = [\varepsilon_1 \ldots \varepsilon_n] \in (0, 1)^n \). It follows from (2.1) that \( \varepsilon \) satisfies

\[
\bar{u}_{i-1} \varepsilon_{i-1} (1 - \varepsilon_i) = \bar{u}_i \varepsilon_i (1 - \varepsilon_{i+1}), \quad i = 1, \ldots, n.
\]

(2.3) with \( \varepsilon_0 := 1 \) and \( \varepsilon_{n+1} := 0 \).

### 2.2. Gain of entrainment in the ribosome flow model

Fix positive numbers \( \bar{u}_i, i = 0, \ldots, n \). We compare two cases. In the first case, we apply the constant control \( u_i(t) = \bar{u}_i, i = 0, \ldots, n \). Then the RFM state variables converge to an equilibrium \( \varepsilon = [\varepsilon_1 \ldots \varepsilon_n] \in (0, 1)^n \) satisfying (2.3). In particular, the protein production rate \( R(t) \) converges to

\[
R_C := \bar{u}_C \varepsilon_C,
\]

where the subscript \( C \) stands for constant.

In the second case, we fix positive and \( T \)-periodic controls \( u_i(t) \), such that \( (1/T) \int_0^T u_i(t) \, dt = \bar{u}_i \). This constraint implies that we invest, on average, the same ‘control effort’ as in the case of constant controls. Then the RFM state variables converge to a unique \( T \)-periodic solution \( \gamma \). Figure 2 depicts the solutions \( x_i(t) \) for the (arbitrarily chosen) initial condition \( x(0) = [0.3 0.4 0.5] \). It may be seen that every \( x_i(t) \) converges to a \( 2\pi \)-periodic solution \( \gamma \). The convergence to this \( \gamma \) holds for any initial condition \( x(0) \in [0, 1]^3 \).

In particular, if the transition rates are set to constant values \( u_i(t) = \bar{u}_i \) for all \( i \) then the RFM admits a unique \( \gamma \)-periodic equilibrium point \( \varepsilon = [\varepsilon_1 \ldots \varepsilon_n] \in (0, 1)^n \). It follows from (2.1) that \( \varepsilon \) satisfies

\[
\bar{u}_{i-1} \varepsilon_{i-1} (1 - \varepsilon_i) = \bar{u}_i \varepsilon_i (1 - \varepsilon_{i+1}), \quad i = 1, \ldots, n.
\]

(2.3) with \( \varepsilon_0 := 1 \) and \( \varepsilon_{n+1} := 0 \).

### 3. Main results

In this section, we analyse several scenarios where the \( n \)-dimensional RFM admits no \( \gamma \) for any periodic control and any period \( T > 0 \).

**Theorem 3.1.** Fix an arbitrary \( T > 0 \). Consider the \( n \)-dimensional RFM with positive and jointly \( T \)-periodic controls \( u_i(t), i = 0, \ldots, n \). If any of the following six conditions holds then there is no \( \gamma \).

\[(i) \text{ } n \text{ is odd, and for all } i \in \{1, 3, \ldots, n - 2\} \text{ there exists } \alpha_i > 0 \text{ such that } u_i(t) = \alpha_i u_{i+1}(t), \text{ for all } t \in [0, T), \] (3.1)
Note that as $x$ converges to the unique $T$-periodic solution $y$, $z$ converges to the unique $T$-periodic solution $γ−e$. Our analysis is based on computing integrals of $(d/dt)z(t)$ and $(d/dt)z^2(t)$ along this periodic solution. To simplify the notation, we write $\int_0^T (d/dt)(z(t))^k dt, k \in \{1, 2\}$, but we always integrate along the unique $T$-periodic solution $γ$. It is useful to introduce the following notation. For a $T$-periodic function $y(t)$, let

$$\bar{y}: = \frac{1}{T} \int_0^T y(t) dt,$$

i.e. the average of $y(t)$ along a period. Note that $\bar{z}_i = \bar{x}_i - e_i$, so $\bar{z}_i > 0$ if and only if (iff) the average of $x_i$ for the periodic transition rates is larger than the equilibrium value $e_i$ corresponding to the constant rates. We also denote

$$\eta_{ij} := \bar{\eta}_i \bar{\eta_j}^2; \quad \eta_{i,j} := \bar{\eta}_i \bar{\eta}_j \bar{\eta}_k,$$

where, as noted above, the averages are always computed along the unique periodic solution. Recall that $z(t) = z_n + \eta(t) \equiv 0$, so any ‘moment’ $\eta$ that includes $\bar{z}_0$ or $\bar{z}_n+1$ (e.g. $\eta_{n,n+1} = \bar{\eta}_n \bar{\eta}_{n+1}$) is zero.

The next result uses the compartmental structure of the RFM to derive a simple necessary and sufficient condition guaranteeing that there is no GOE.

**Proposition 3.2.** We have

$$\eta_{0,1} = -\eta_{n,n} \quad (3.7)$$

and

$$R_p = -\eta_{0,1} + R_C \quad (3.8)$$

In particular, if $\eta_{0,1} \geq 0$ then there is no GOE, and if $\eta_{0,1} > 0$ then $R_p$ is strictly smaller than $R_C$.

**Remark 3.3.** Note that $\eta_{n,n} = \bar{\eta}_n$ may be interpreted as the ‘average correlation’ between the initiation rate $u_0$ and the centred density $z_1 = x_1 - e_1$. Note also that (3.7) implies there exists a time $\tau \in [0, T)$ such that

$$u_0(\tau)z_1(\tau) = -u_1(\tau)z_n(\tau).$$

**Proof.** It follows from (3.6) that

$$z_1 + \cdots + z_n = u_0(1 - z_1 - e_1) - u_n(z_n + e_n).$$

Integrating this equation gives

$$0 = \bar{u}_0(1 - e_1) - \eta_{0,1} - \bar{u}_n - \bar{\eta}_{n,n},$$

and using (2.3) proves (3.7). To complete the proof note that

$$R_p = \bar{u}_0 \bar{\eta}_n = \bar{u}_n(z_n + e_n) = \bar{\eta}_{n,n} + R_C,$$

and applying (3.7) yields (3.8).
Proposition 3.4. For any \( i \in \{0, \ldots, n\} \), we have
\[
\eta_{i,i+1} = \eta_{i,1} + \eta_{i}(1 - c_{i+1}) - \eta_{i,i+1}c_{i}. \tag{3.9}
\]

Proof. First, we prove (3.9) for \( i = 0 \) and \( i = n \). For \( i = 0 \), (3.9) becomes
\[
0 = \eta_{0,1} + \eta_{0} - \eta_{0,1}c_{0},
\]
which clearly holds. Similarly, for \( i = n \), we get
\[
0 = \eta_{n,1} + \eta_{n},
\]
which holds due to proposition 3.2.

In the rest of the proof we assume that \( i \in \{1, \ldots, n - 1\} \). Integrating (3.6) and using (2.3) gives
\[
0 = u_{i-1}(1 - c_{i} - z_{i})(z_{i-1} + c_{i-1}) - u_{i}(z_{i} + c_{i})(1 - c_{i+1} - z_{i+1}) - \eta_{i-1,i-1} + \eta_{i-1,i-1}(1 - c_{i} - z_{i-1}) - \eta_{i-1,i-1}c_{i-1} + \eta_{i,i+1}c_{i}.
\]
(3.10)

For \( i = 1 \), this becomes
\[
\eta_{1,2} = \eta_{1,1} + \eta_{1,1}(1 - c_{2}) - \eta_{1,2}c_{1} \tag{3.11}
\]
(recall that \( z_{2}(t) = z_{0}(t) \equiv 0 \)). For \( i = 2 \), equation (3.10) becomes
\[
\eta_{2,2,3} = \eta_{2,2} - \eta_{2,1}(1 - c_{2}) + \eta_{2,2}c_{2} + \eta_{2,3}(1 - c_{3}) - \eta_{2,3}c_{2},
\]
and using (3.11) gives
\[
\eta_{2,2,3} = \eta_{2,1} + \eta_{2,2}(1 - c_{3}) - \eta_{2,3}c_{2}.
\]
Continuing in this manner completes the proof. 

The next result is derived by integrating \( \frac{1}{2}(d/dt)z_{i}^{2}(t) = z_{i}(t)\dot{z}_{i}(t) \), \( i = 1, \ldots, n \). This yields \( n \) lower bounds on \( \eta_{0,1} \).

Proposition 3.5. For any \( i \in \{1, \ldots, n\} \), we have
\[
\eta_{0,1} \geq \eta_{i,1}c_{i}^{2} - \eta_{i-1,i}(1 - c_{i})^{2}. \tag{3.12}
\]
Furthermore, equality is attained iff \( z_{i}(t) \equiv 0 \), that is, iff \( x_{i}(t) \equiv c_{i} \).

Proof. By (3.6),
\[
z_{i}z_{i} = u_{i-1}z_{i}(1 - c_{i} - z_{i})(z_{i-1} + c_{i-1}) - u_{i}z_{i}(z_{i} + c_{i})
\times (1 - c_{i+1} - z_{i+1}).
\]
Integrating this and rearranging terms gives
\[
(u_{i-1}(z_{i-1} + c_{i-1}) + u_{i}(1 - c_{i+1} - z_{i+1}))z_{i} = u_{i-1}z_{i}(1 - c_{i})(z_{i-1} + c_{i-1}) - u_{i}z_{i}(1 - c_{i+1} - z_{i+1}).
\]
(3.13)
The left-hand side of this equation is non-negative because the controls \( u_{i} \) are positive for all \( t \), and along the periodic solution \( z_{i}(t) + c_{i} = \gamma_{i}(t) \equiv 0 \) for all \( t \). Furthermore, the left-hand side is zero iff \( z_{i}(t) \equiv 0 \). We conclude that
\[
0 \leq \eta_{i-1,i-1}(1 - c_{i}) + \eta_{i-1,i}(1 - c_{i})c_{i-1} + \eta_{i,i+1}c_{i}
- \eta_{i}(1 - c_{i+1})c_{i},
\]
with equality iff \( z_{i}(t) \equiv 0 \), and substituting the expressions for the third-order moments (3.9) completes the proof. 

Note that for \( i = 1 \) and \( i = n \), proposition 3.5 yields
\[
\eta_{0,1} \geq \eta_{i,1}c_{i}^{2} \tag{3.15}
\]
and
\[
\eta_{0,1} \geq -\eta_{i-1,i-1}(1 - c_{i})^{2},
\]
respectively. Combining this with proposition 3.2 implies that there is no GOE if \( \eta_{2,2} \geq 0 \) or if \( \eta_{n-1,n-1} \leq 0 \).

For \( n = 1, z_{2}(t) \equiv 0 \), so \( \eta_{2,2} = \eta_{1,1} = 0 \) and (3.15) becomes \( \eta_{0,1} \geq 0 \). Combining this with proposition 3.2 yields the following result.

Corollary 3.6. In the RFM with \( n = 1 \), there is no GOE. Furthermore, \( R_{p} = R_{C} \iff \)
\[
u_{1}(t) = \frac{\eta_{1}}{\eta_{0}}u_{0}(t), \quad \text{for all } t \in [0, T].
\]
(3.17)

Proof. The proof that \( R_{p} \leq R_{C} \) follows immediately from proposition 3.2. Now assume that \( R_{p} = R_{C} \). Then \( z_{2}(t) \equiv 0 \) along the unique periodic solution, so
\[
x_{1}(t) = c_{1} = \frac{\eta_{0}u_{0}(t) - \eta_{0}u_{1}(t)}{\eta_{0} + \eta_{1}}.
\]
Substituting this in (2.1) yields
\[
0 = u_{0}(t)(1 - c_{1}) - u_{1}(t)c_{1} = \frac{\eta_{1}u_{0}(t) - \eta_{1}u_{1}(t)}{\eta_{0} + \eta_{1}},
\]
and this completes the proof. 

The fact that in the one-dimensional RFM there is no GOE was already proved in [41] using a more complicated argument (see also [42,60]).

We can now prove theorem 3.1 for \( n \geq 2 \). To prove assertion (i), suppose that \( n \) is odd, i.e. \( n = 2k + 1 \), and that all the internal rates are proportional in pairs, that is, for any \( i \in \{1, 3, \ldots, n - 2\} \) there exists \( \alpha_{i} > 0 \) such that
\[
u_{i}(t) = \alpha_{i}u_{i+1}(t), \quad \text{for all } t \in [0, T].
\]
(3.18)
For \( i = 1 \), equation (3.12) becomes
\[
\eta_{0,1} \geq \eta_{2,2}c_{2}^{2} - \eta_{2,2}(1 - c_{2})^{2},
\]
so if \( \eta_{2,2} \geq 0 \) then there is no GOE and the proof is completed. We may thus assume that \( \eta_{2,2} < 0 \). For \( i = 3 \), equation (3.12) becomes
\[
\eta_{0,1} \geq \eta_{3,2}c_{2}^{2} - \eta_{3,2}(1 - c_{2})^{2},
\]
and using (3.18) gives \( \eta_{0,1} \geq \alpha_{3}\eta_{1}c_{2}^{2} - \eta_{2,2}(1 - c_{2})^{2} \). Thus, if \( \eta_{2,2} \geq 0 \) then there is no GOE, and we may assume that \( \eta_{4,4} \leq 0 \). Continuing in this manner, we may assume that \( \eta_{2,2} < 0 \) for all \( j \leq k \). For \( i = n = 2k + 1 \), equation (3.12) becomes
\[
\eta_{0,1} \geq -\eta_{2,2}(1 - c_{2k+1})^{2} > 0,
\]
and this completes the proof of assertion (i) in theorem 3.1.

To prove assertion (ii), suppose that \( n \) is even and that (3.2) holds. Then \( \eta_{0,1} = \alpha_{1}\eta_{1,1} \), and we have established no GOE if \( \eta_{1,1} \geq 0 \). Therefore, we may assume that \( \eta_{1,1} < 0 \). Considering (3.12) with \( i = 2 \), we conclude that there is no GOE if \( \eta_{2,2} = \alpha_{2}\eta_{2,2} \geq 0 \), and we may assume that \( \eta_{3,3} < 0 \). Continuing in this manner, we have that \( \eta_{n-1,n-1} < 0 \), and we already know from inequality (3.16) that this implies that there is
no GOE. This completes the proof of assertion (ii) in theorem 3.1.

To prove assertion (iii), suppose that \( n \) is even and that (3.3) holds. Then \( \eta_{n-1,a} = \alpha_{n-1} \eta_{n,a} \) and due to proposition 3.2 there is no GOE if \( \eta_{n-1,a} \leq 0 \). Therefore, we may assume that \( \eta_{n-1,a} > 0 \). Considering (3.12) with \( i = n - 1 \) we conclude that there is no GOE if \( \eta_{n-2,n-2} = \alpha_{n-2} \eta_{n-3,n-2} \leq 0 \), and we may assume that \( \eta_{n-3,n-2} > 0 \). Continuing in this manner, we have that \( \eta_{1,2} > 0 \), and we already know from inequality (3.15) that this implies that there is no GOE. This completes the proof of assertion (iii) in theorem 3.1.

Assertions (iv)–(vi) follow immediately from assertions (i)–(iii), respectively, as one may choose \( a_i = \pi_i/\pi_{i+1} \) for the corresponding values of \( i \). This completes the proof of theorem 3.1.

Reconsidering the proof of proposition 3.5 allows us to slightly strengthen theorem 3.1. To do this, we introduce another definition.

**Definition 3.7.** A \( T \)-periodic solution \( \gamma : [0, T) \rightarrow (0, 1)^n \) of the RFM is called degenerate if there exists an index \( i \in \{1, \ldots, n\} \) such that \( \gamma(t) \) is constant. Otherwise, \( \gamma \) is called non-degenerate.

**Corollary 3.8.** Suppose that one of the cases in theorem 3.1 holds. Then for any \( T \)-periodic control \( u(t) \) that yields a non-degenerate \( T \)-periodic solution we have

\[
R_p < R_C. \tag{3.19}
\]

In other words, in this case, constant rates are in fact strictly better than periodic ones.

**Proof.** Fix an arbitrary \( i \in \{1, \ldots, n\} \). Since the periodic solution is non-degenerate, \( z_i(t) = x_i(t) - c_i \) is not zero at some time \( t_i \in [0, T) \), and this implies that the term on the left-hand side of (3.13) is positive. Thus, the inequality in (3.14) becomes strict. Now the arguments in the proof of theorem 3.1 yield (3.19).

While theorem 3.1 proves that there is no GOE in certain cases, numerical experiments suggest that there is no GOE in general.

**Example 3.9.** We simulated an RFM with \( n = 4 \). Figure 3 shows the moving average of the production rate

\[
\int_{t_j}^{t_j + T} u_4(\tau)x_4(\tau) \, d\tau
\]

for several simulations. The average value of the positive transition rates was kept the same in all simulations, and the initial conditions were always set to the steady state corresponding to the constant inputs, that is, \( x(0) = c \). The transition rates were all of the form \( u_i(t) = \pi_i + A_i \cos(2\pi t + \phi_i) \), where \( \phi_i \in [0, 2\pi) \) and \( A_i \in (0, \pi_i) \) were chosen at random. Thus, the rates are \( T \)-periodic, with \( T = 1 \). It can be seen that the average production rate in the periodic case is always lower than the production rate for constant inputs, i.e. \( R_p < R_C \).

Figure 4 depicts \( \gamma_4(t) \) over a single period in the simulations.

**Figure 3.** Moving average of the production rate \( u_4x_4 \) in an RFM with \( n = 4 \) for different choices of 1-periodic transition rates.

**Figure 4.** Plot of \( \gamma_4(t) \) over a single period for several simulations using different transition rates with the same average values.

Solid lines show the instantaneous value and dashed lines show the average over a single period. The black solid line is the value \( c_4 \). Note that in some cases \( \gamma_4 > c_4 \) while in others \( \gamma_4 < c_4 \). In all cases, \( \gamma_4 \) oscillates around \( c_4 \), so \( z_4(t) = \gamma_4(t) - c_4 \) changes sign.

The mean values of the inputs used in the simulations are \( \bar{\pi}_1 = 13.56, \bar{\pi}_2 = 11.38, \bar{\pi}_3 = 3.90, \bar{\pi}_4 = 3.53 \) and \( \bar{\pi}_5 = 2.34 \). The parameters \( A_i, \phi_i \) of all inputs in each of the simulations are included in table 1.

### 4. Discussion

We studied the GOE in the \( n \)-dimensional RFM. Our analysis approach is new and is based on calculating integrals of the (centred) state variables and ‘higher-order moments’ of these variables along the periodic solution.

We proved that under certain conditions the \( n \)-dimensional RFM admits no GOE. We conjecture that this in fact always true, but even for the case \( n = 2 \), we are not able to prove this in the most general setting.
An intuitive explanation for the fact that constant controls are always optimal, based on numerical simulations, is as follows. When $u(t)$ is $T$-periodic and non-constant there are times $t$ such that $u(t) > \bar{u}$ and times when the opposite inequality holds. In general, the ‘profit’ to the average production rate in the ‘good’ times is less than the ‘loss’ in the ‘bad’ times. An interesting line for further research is to try and rigorously formulate and prove this observation. Note that in the case of optimal periodic control for scalar systems (i.e. when $n = 1$), convexity theory plays an important role [51,52].

One case where we proved that there is no GOE is when $n$ is odd and all the internal rates are equal. It is interesting to note that in TASEP, with all rates constant, the case of equal internal hopping rates is the one that is amenable to analysis. Note also that it was recently shown that TASEP just like the RFM, is a contractive system (in a suitable stochastic sense) [61].

Finally, entrainment to periodic excitations is important in many natural and artificial systems. For example, connecting several artificial biological systems that entrain to a common clock may lead to a well-functioning modular system [63]. Epidemic outbreaks entrain to seasonal forcing [64]. Analysis of GOE in such models is related to interesting questions like: (1) is periodic production of synthetic biology constructs more efficient than constant production? and (2) does seasonality make the epidemics, on average, more or less severe?

### Data accessibility
This article has no additional data.

### Authors’ contributions
R.O.: conceptualization, investigation, writing—original draft, writing—review and editing; T.K.: conceptualization, investigation, writing—original draft, writing—review and editing; L.G.: conceptualization, investigation, writing—original draft, writing—review and editing; M.M.: conceptualization, investigation, writing—original draft, writing—review and editing.

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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### Endnote
1All the simulations in this paper were performed using MATLAB, and all numerical values are to four-digit accuracy.

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