



A max-plus model of ribosome dynamics during mRNA translation

Chris A. Brackley^{a,*}, David S. Broomhead^b, M. Carmen Romano^{a,c}, Marco Thiel^a

^a Institute for Complex Systems and Mathematical Biology, SUPA, University of Aberdeen, Aberdeen AB24 3UE, UK

^b School of Mathematics, Alan Turing Building, The University of Manchester, Oxford Road, Manchester M13 9PL, UK

^c Institute of Medical Sciences, Foresterhill, University of Aberdeen, Aberdeen AB25 2ZD, UK

ARTICLE INFO

Article history:

Received 11 October 2011

Received in revised form

21 February 2012

Accepted 7 March 2012

Available online 16 March 2012

Keywords:

Protein biosynthesis

Elongation

Petri net

Tropical algebra

ABSTRACT

We examine the dynamics of the translation stage of cellular protein production, in which ribosomes move uni-directionally along an mRNA strand, building amino acid chains as they go. We describe the system using a timed event graph—a class of Petri net useful for studying discrete events, which have to satisfy constraints. We use max-plus algebra to describe a deterministic version of the model, where the constraints represent steric effects which prevent more than one ribosome reading a given codon at a given time and delays associated with the availability of the different tRNAs. We calculate the protein production rate and density of ribosomes on the mRNA and find exact agreement between these analytical results and numerical simulations of the deterministic model, even in the case of heterogeneous mRNAs.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Messenger RNA (mRNA) translation is one of the steps in protein production in cells (Alberts et al., 2008). The mRNAs are single strands of nucleotides which are transcribed from the DNA. The sequence of nucleotides, grouped in triplets called codons, holds the code for a specific chain of amino acids that makes up a protein. Translation is performed by molecular machines called ribosomes, which scan along the mRNA adding amino acids to a growing chain which will become the protein. The rates at which different proteins are produced are crucial in determining how a cell grows and functions.

Various statistical models have been used to describe and understand the translation process. For example, the totally asymmetric simple exclusion process (TASEP) is a “driven diffusion” system where particles representing the ribosomes move along a lattice which represents the mRNA molecule (MacDonald et al., 1968; Derrida et al., 1992; Shaw et al., 2003; Dong et al., 2007). TASEP based models offer rich dynamics and are well studied in the non-equilibrium physics literature; they provide a simple, yet powerful approximation of the dynamics of ribosomes on mRNAs. Other approaches include using ODE modelling methods to give a mean field approximation (Elf et al., 2001; Elf and Ehrenberg, 2005; Gilchrist and Wagner, 2006). This involves using master equations to determine the probability of finding a

ribosome translating a given part of an mRNA, but largely ignoring correlations and stochastic effects.

In this paper, we propose a new model, which can be analysed rather completely using algebra on the max-plus semi-ring. The most convenient way to present the model is to write it as a deterministic Petri net (Murata, 1989). This can then be analysed completely – and exactly – as a linear dynamical system, when written in terms of max-plus algebra (Heidergott et al., 2006). The model gives a more realistic representation of the movement of ribosomes than that of many previous studies and explicitly considers the exact genetic coding sequence, as well as the fact that different codons are translated at different rates. We perform numerical simulations of the Petri net, and find that the results do, indeed, match exactly the behaviour predicted by the max-plus analysis.

Although the general sequence of events in translation is understood, there are many questions which remain unanswered. Translation begins when a ribosome binds to the end of an mRNA strand – a process known as initiation. In this paper, we focus on the second stage of translation, which is known as elongation. Here, the ribosome moves uni-directionally along the mRNA, pausing at each codon to recruit an amino acid, which is then added to the growing chain. Amino acids are transported within the cytoplasm by carrier molecules called transfer RNAs (tRNAs). Different species of tRNA carry different amino acids, and different codons correspond to different tRNAs. The different tRNAs appear in different concentrations, so the time which a ribosome waits for the required tRNA to arrive differs from codon to codon (Robinson et al., 1984; Sørensen et al., 1989). Since it is often the case that several ribosomes are bound to the mRNA at the same

* Corresponding author.

E-mail address: c.a.brackley@abdn.ac.uk (C.A. Brackley).

time, the distribution of waiting times can result in traffic jams if the progress of one ribosome is obstructed by another.

This picture is complicated by the fact that whilst there are only 20 common amino acids, in the model organism *Saccharomyces cerevisiae* there are 41 species of tRNA, i.e. there is redundancy in the genetic code. For some amino acids there are multiple tRNAs; furthermore, it is often the case that there is a highly abundant tRNA and a rare tRNA which code for the same amino acid. Sometimes a “slow codon”, which will cause a long pause in elongation, is used even though a fast codon corresponding to the same amino acid is available. The interest for biologists is therefore to understand how slow codons affect protein production rates and the use of ribosomes (Brockmann et al., 2007).

Translation reaches its completion in the termination stage when a ribosome encounters a “stop” codon, where, through the binding of release factors, the ribosome disassociates from the mRNA and the amino acid chain is released ready for further folding or processing. The quantities of interest which we shall take from the model are the time interval between successive amino acid chains being completed, which we will call the protein production time, and the ribosome occupation density of the mRNA.

In the next section, we introduce Petri nets, and detail how these can be used to describe translation. Then in Section 3 we introduce the max-plus semi-ring, giving a brief survey of its salient properties required in the rest of the paper. In Section 4 we detail the max-plus treatment of the Petri net describing translation. These results are then compared to numerical simulations: we first consider simple “designer mRNAs” where each codon corresponds to the same tRNA, before treating realistic sequences taken from the *S. cerevisiae* genome. Finally, we consider how introducing stochasticity into the model is likely to alter the results, and how the present work relates to previous models of translation (specifically the TASEP).

2. Timed Petri nets

Petri nets (attributed to Petri, 1962) are a scheme whereby a sequence of discrete events is described on a network. We give a brief introduction here, but for a detailed description refer to Murata (1989) and references therein.

A Petri net is a directed graph which contains two types of nodes: places and transitions. These are shown diagrammatically in Fig. 1(a). Directed arcs connect places with transitions but not

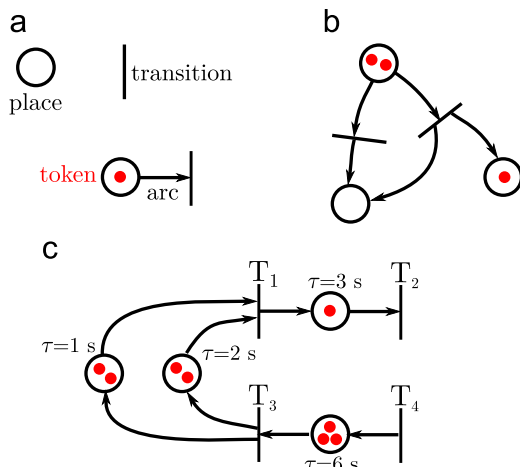


Fig. 1. (a) Petri net components: places, transitions, arcs and tokens. (b) A simple Petri net. (c) An example timed Petri net with transitions labelled $T_1, \dots, T_4$, and different waiting times τ assigned to each place.

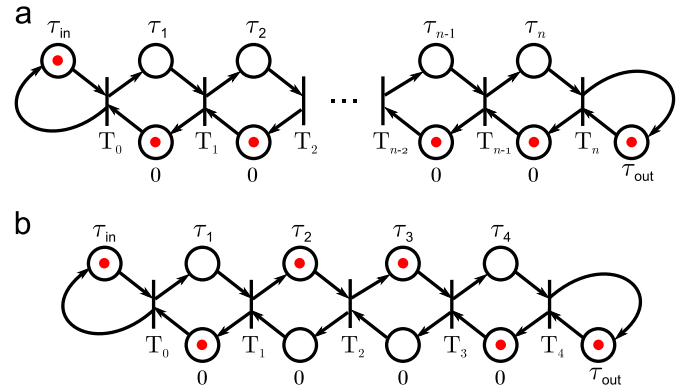


Fig. 2. (a) A Petri net describing mRNA translation. Each pair (upper and lower) of places represents one codon. Tokens (red) in the bottom row represent vacant codons; in the top row they represent a ribosome reading that codon. The initial condition with no ribosomes on the mRNA is shown. (b) An example Petri net for a short mRNA of length $n=4$ codons (plus the stop codon), with a token configuration representing ribosomes occupying codons 2 and 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

places with places or transitions with transitions. Petri nets are therefore bipartite graphs.

Petri nets have additional structure which allows them to model discrete event processes. To this end, places can contain objects called tokens. A transition is said to be *active* when each of the adjacent upstream places contains at least one token. Fig. 1(b) shows an example. When a transition is active it can *fire*. Upon firing, one token is removed from all adjacent places upstream of the transition and one token is added to all adjacent places downstream. Clearly, there is no implicit conservation of tokens since the number of upstream places need not equal the number of downstream places. Events unfold in a discrete manner. It is usual, when using Petri nets to model systems of discrete events, to associate each event with a transition firing. The conditions required for an event to occur are then represented by the presence or lack of tokens in the upstream adjacent places.

A timed Petri net is an extension of this framework in which a waiting time is attached to each place. Fig. 1(c) shows an example. Whenever a token is put into a place, it will only contribute to the activation of transitions once a time τ associated with that place has elapsed. This can be thought of as a timer on the token which is started as it enters a place. As soon as a transition becomes active it fires. In this way, the Petri net describes discrete events occurring in continuous time as conditions are fulfilled. Unlike some other models (for example, Monte Carlo based simulations using a random update rule) events can occur simultaneously.

We can describe the movement of ribosomes along the mRNA as a timed Petri net and this is shown in Fig. 2(a). A sequence of codons of length n is represented by two rows of places, with each pair (upper and lower) representing a codon. A token in the top row indicates that a ribosome is decoding that codon, and a token in the bottom row indicates a vacant codon. Having this two row structure ensures that there can only be one token in each place, i.e. one ribosome translating each codon. This, of course, requires that the initial state also satisfies this condition.¹ A suitable initial state is having all top row places empty and all bottom row places

¹ This structure has a small cycle corresponding to each codon. It is easy to show that the number of tokens in each of these cycles is conserved.

containing one token; this corresponds to an mRNA initially free of ribosomes.

The firing of a transition T_i represents the movement of a ribosome from codon i to codon $i+1$. There are $n+1$ transitions, labelled $i=0, \dots, n$, with the firing of the zeroth and n th transitions representing initiation and termination events, respectively. At the leftmost end of the Petri net we have only an upper row place and this contains a token; this represents a continuous supply of ribosomes ready to begin translation. Similarly, at the rightmost end of the Petri net there is only a lower row place and this too contains a token. It represents the stop codon and the time it takes to release the ribosome back into the cytoplasm, which is always ready to accept ribosomes. These places are kept full with a token via an arc to and from the same transition.

The time associated with each upper row place corresponds to the time it takes a ribosome to capture a tRNA of the species corresponding to that codon. Generally, the arrival times of tRNAs can be represented by a set of independent Poisson processes, however, a deterministic version of the model can be obtained by replacing these random times with their means. In the present paper, we concentrate on the deterministic version; the consequences of introducing stochasticity are discussed in Section 6. We assume that the mean codon waiting time is proportional to the concentration of the species of tRNA corresponding to that codon. We denote the waiting time for the i th codon counting from the left τ_i . We assume that when a ribosome moves forward the codon it was covering is immediately vacant, and the time it takes for the ribosome to physically move is negligible; thus, we associate a time zero with each of the lower row of places. In reality a ribosome actually covers more than just the codon it is translating but for simplicity we do not take this into account here. The first place in the chain – corresponding to initiation – has a time τ_{in} associated with it. Biologically, this corresponds to the average time interval between attempts for a ribosome to begin translation, and will depend on the availability of ribosomes and various initiation factors, as well as the presence of any secondary structures in the non-coding leader region of the mRNA. Since our focus here is on elongation, we assume that τ_{in} is constant in time, treating it as a control parameter. The final place in the chain has an associated time τ_{out} , which is the time a ribosome waits at the stop codon at end of the mRNA before releasing the completed protein and detaching from the mRNA. Biologically, this could be linked to the availability of a number of release factors, and again we treat it as a control parameter. The model is therefore parametrised by the initiation and termination waiting times τ_{in} and τ_{out} , and the set of internal tRNA capture waiting times $\{\tau_i | 1 \leq i \leq n\}$.

Fig. 2(b) shows an example Petri net for a very (unrealistically) short mRNA of length $n=4$ codons, with ribosomes occupying codons 2 and 3. Transition T_2 cannot fire because there is no token in its input place in the lower row (representing a vacancy). Transition T_3 will fire as soon as the token in its upper row input place has been there for a time τ_3 . If the token in the place upstream of T_2 (upper row place for codon 2) has been there for a time longer than τ_2 when T_3 fires, then T_2 will also fire at this time. Thus, if there is a slow codon (large τ_i), when the corresponding transition fires, it could result in several other transitions firing simultaneously.

3. The max-plus semi-ring

If each place in a Petri net has exactly one upstream and one downstream adjacent transition (as is the case in those depicted in Fig. 2), then its dynamics can be described using max-plus algebra. Max-plus is an algebra over the semi-ring $\mathbb{R}_{\max} = \mathbb{R} \cup -\infty$. In this

section, we give a brief overview of the theorems required in the rest of the paper and refer the reader to Heidergott et al. (2006) and Cohen et al. (1999) for further details and proofs.

In max-plus algebra the addition and multiplication operators $\oplus$ and $\otimes$ are defined as

$$a \oplus b = \max(a, b) \quad \text{and} \quad a \otimes b = a + b,$$

where $a, b \in \mathbb{R}_{\max}$. Max-plus algebra refers to $\mathcal{R}_{\max} = (\mathbb{R}_{\max}, \otimes, \oplus, \epsilon, e)$, i.e. the elements of $\mathbb{R}_{\max}$ together with the binary operations $\otimes$ and $\oplus$. Here, $\epsilon = -\infty$ and $e = 0$ behave as zero and unity, respectively. This algebra has many of the same properties as conventional algebra, e.g.

$$a \otimes b = b \otimes a,$$

$$a \otimes (b \oplus c) = a \otimes b \oplus a \otimes c,$$

$$a \otimes \epsilon = \epsilon,$$

$$a \otimes e = a, \quad \text{etc.}$$

We define max-plus powers in the natural way

$$a^{\otimes k} = a \otimes a \otimes \dots \otimes a \quad k \text{ times}$$

and note that in conventional algebra this corresponds to $k \times a$. We also note that the $\otimes$ operator has an inverse which can be expressed as a negative power

$$a \otimes b^{\otimes(-1)} = a - b$$

but there is no inverse of the $\oplus$ operator.

Vectors and matrices can also be constructed; we denote by $[A]_{ij}$ the i - j th component of the matrix A . For matrices $A \in \mathbb{R}_{\max}^{n \times m}$, $B \in \mathbb{R}_{\max}^{m \times l}$ and $C \in \mathbb{R}_{\max}^{m \times l}$ the sum and product of matrices are then defined as

$$[A \oplus B]_{ij} = a_{ij} \oplus b_{ij},$$

$$[A \otimes C]_{ij} = \bigoplus_{k=1}^m a_{ik} \otimes c_{kj},$$

where $a_{ij} = [A]_{ij}$, $b_{ij} = [B]_{ij}$ and $c_{ij} = [C]_{ij}$, and we have used the following convenient notation for summation over indices:

$$\bigoplus_{i=1}^n a_i = \max\{a_i | 1 \leq i \leq n\}.$$

Square matrices can have associated eigenvectors and eigenvalues, i.e. for $A \in \mathbb{R}_{\max}^{n \times n}$ a vector $\mathbf{u} \in \mathbb{R}_{\max}^n$ satisfying

$$A \otimes \mathbf{u} = \lambda \otimes \mathbf{u}$$

is an eigenvector of A associated with eigenvalue λ . As with conventional linear algebra, eigenvectors are not unique, thus if $\mathbf{u}$ is an eigenvector then so is $\alpha \otimes \mathbf{u}$ with α an arbitrary finite number. In general, a matrix can have multiple associated eigenvalues.

The properties of a max-plus matrix can often be determined by considering the associated directed weighted graph. The graph associated with matrix $A \in \mathbb{R}_{\max}^{n \times n}$, denoted $\mathcal{G}(A)$, consists of a set of nodes and directed weighted arcs, where if there is a matrix element $[A]_{ij}$ with value $a_{ij} \neq \epsilon$, then there is an arc from node $j \rightarrow i$ with weight a_{ij} (note this slightly unusual convention for the direction of the arc). A series of one or more arcs between two nodes i and j is called a *path* from $i \rightarrow j$. A path $i \rightarrow i$ is called a *circuit* and denoted γ . A graph is said to be *strongly connected* if for any two different nodes there is a path between them and a matrix A is said to be irreducible if the corresponding graph $\mathcal{G}(A)$ is strongly connected.

The *circuit weight* w_γ of a circuit γ is defined as the sum of the weights of all arcs in that circuit and the *circuit length* l_γ as the number of arcs in the circuit. The *mean circuit weight* is then

defined as $\bar{w}_\gamma = w_\gamma / l_\gamma$. If the maximum mean circuit weight in a graph is λ , then a circuit with a mean circuit weight equal to λ is called a *critical circuit*. The *critical graph* corresponding to matrix A , denoted $\mathcal{G}^c(A)$, is defined as the subgraph of $\mathcal{G}(A)$ containing only the nodes and arcs which are in the critical circuits. The cyclicity of a strongly connected graph $\sigma_{\mathcal{G}}$ is defined as the greatest common divisor of the lengths of all of the circuits in that graph. If the graph is not strongly connected, its cyclicity is equal to the least common multiple of the cyclicities of each of its maximal strongly connected subgraphs. The cyclicity of a matrix A , σ_A , is equal to the cyclicity of the critical graph of A , $\mathcal{G}^c(A)$.

Theorem 1. Any irreducible matrix $A \in \mathbb{R}_{\max}^{n \times n}$ possesses one and only one eigenvalue λ , which is a finite number equal to the maximal mean circuit weight of circuits in the graph $\mathcal{G}(A)$. (See, for example, Theorem 2.9 in Heidergott et al., 2006 for proof.)

Thus, the eigenvalue of an irreducible matrix can be found by considering the corresponding graph.

As noted above there is a whole continuum of eigenvectors associated with the eigenvalue of a max-plus matrix. Two eigenvectors $\mathbf{x}, \mathbf{y}$ are *co-linear* if there exists a scalar $\alpha \in \mathbb{R}_{\max}$ such that $\mathbf{y} = \alpha \otimes \mathbf{x}$, and such vectors can be projected onto the same object in a projective space (Heidergott et al., 2006). A given eigenvalue of the matrix A can be associated with more than one linearly independent eigenvector and the number of such eigenvectors can be found by considering the critical graph $\mathcal{G}^c(A)$.

Theorem 2. If the critical graph $\mathcal{G}^c(A)$ of an irreducible matrix $A \in \mathbb{R}_{\max}^{n \times n}$ has k maximal strongly connected subgraphs, then A has k linearly independent eigenvectors. (For proof see, for example, Theorem 4 in Cohen et al., 1985.)

We shall see below that the problem studied in this paper involves sequences of vectors $\mathbf{x}(k) \in \mathbb{R}_{\max}^n, k \in \mathbb{N}$ described by a recurrence equation

$$\mathbf{x}(k) = A \otimes \mathbf{x}(k-1)$$

or equivalently

$$\mathbf{x}(k) = A^{\otimes k} \otimes \mathbf{x}(0) \quad (1)$$

for $k \geq 0$, where $A \in \mathbb{R}_{\max}^{n \times n}$ is irreducible and $\mathbf{x}(0)$ is some initialisation vector. The following theorem is central to the understanding of these sequences.

Theorem 3 (Cyclicity Theorem). There is an integer K_0 such that

$$A^{\otimes(k+\sigma_A)} = \lambda^{\otimes \sigma_A} \otimes A^{\otimes k}, \quad k \geq K_0,$$

where λ and σ_A are the eigenvalue and cyclicity of the matrix $A \in \mathbb{R}_{\max}^{n \times n}$, respectively. (For proof see Cohen et al., 1985 or Theorem 3.9 in Heidergott et al., 2006.)

This implies some periodicity in the powers of A ; the periodic behaviour is characterised by λ and σ_A , and K_0 is known as the *transient time* of A . By using this in Eq. (1), we find

$$\begin{aligned} \mathbf{x}(k+\sigma) &= A^{\otimes(k+\sigma)} \otimes \mathbf{x}(0) \\ &= \lambda^{\otimes \sigma} \otimes A^{\otimes k} \otimes \mathbf{x}(0) \\ &= \lambda^{\otimes \sigma} \otimes \mathbf{x}(k). \end{aligned}$$

If matrix A has cyclicity $\sigma_A = 1$, then we have

$$\begin{aligned} \mathbf{x}(k+1) &= A \otimes \mathbf{x}(k) \\ &= \lambda \otimes \mathbf{x}(k), \quad k \geq K_0 \end{aligned} \quad (2)$$

i.e. $\mathbf{x}(k)$ (or equivalently $A^{\otimes k} \otimes \mathbf{x}(0)$ for any $\mathbf{x}(0) \in \mathbb{R}_{\max}^n$) is an eigenvector of A for $k \geq K_0$. In this case, the effect of the initial condition disappears after K_0 iterations.

Finally, we turn to linear equations of the form

$$\mathbf{x} = (A \otimes \mathbf{x}) \oplus \mathbf{b}, \quad (3)$$

where $A \in \mathbb{R}_{\max}^{n \times n}$ and $\mathbf{x}, \mathbf{b} \in \mathbb{R}_{\max}^n$. These can often be solved – despite the lack of an obvious way to define the inverse of A – using an object called the Kleene star. This is defined as

$$A^* = \bigoplus_{k=0}^{\infty} A^{\otimes k}. \quad (4)$$

The existence of A^* can be proved if the graph $\mathcal{G}(A)$ has only non-positive circuit weights (Heidergott et al., 2006).

Theorem 4. If $\mathcal{G}(A)$ has maximal mean circuit weight less than or equal to ϵ , then $\mathbf{x} = A^* \otimes \mathbf{b}$ is a unique solution to Eq. (3). (For proof see, for example, Theorem 2.10 in Heidergott et al., 2006.)

4. Analysis of translation with the max-plus algebra

A Petri net can be described using max-plus algebra by defining the matrices $A_0, A_1, \dots, A_M$. These depend on the initial conditions such that the matrix component $[A_m]_{ij}$ is equal to the maximum of the holding times on all places which lie between transitions j and i , and contain exactly m tokens. Where there is no such place, then $[A_m]_{ij} = \epsilon$. For example, if the configuration of the Petri net shown in Fig. 1(c) is taken as the initial condition, we have $M=3$ and $[A_1]_{21} = 3$ s, $[A_2]_{13} = 2$ s, and $[A_3]_{34} = 6$ s, with all other components equal to ϵ . The dynamics are then described by the vector $\mathbf{x}(k)$, where the i th component of the vector is equal to the time at which the i th transition fires for the k th time. This vector satisfies the recursion equation

$$\mathbf{x}(k) = A_0 \otimes \mathbf{x}(k) \oplus A_1 \otimes \mathbf{x}(k-1) \oplus \dots \oplus A_M \otimes \mathbf{x}(k-M).$$

For further details see Heidergott et al. (2006), Baccelli et al. (1992), or Gaubert and Mairesse (1999).

In the case of the Petri net which describes mRNA translation (shown in Fig. 2(a)), the structure and initial conditions are such that each place can contain either 0 or 1 tokens, therefore, in this case $M=1$ and the above equation reduces to

$$\mathbf{x}(k) = A_0 \otimes \mathbf{x}(k) \oplus A_1 \otimes \mathbf{x}(k-1). \quad (5)$$

In the Petri net, we have $n+1$ transitions labelled $0, \dots, n$, so $\mathbf{x}(k)$ has components $\{x_i | 0 \leq i \leq n\}$. Likewise, A_0 and A_1 are $(n+1) \times (n+1)$ matrices, with elements labelled by indices running from 0 to n . For the initial conditions shown in Fig. 2(a), these matrices are

$$A_0 = \begin{pmatrix} \epsilon & \epsilon & \epsilon & \dots & \epsilon \\ \tau_1 & \epsilon & \epsilon & \dots & \epsilon \\ \epsilon & \tau_2 & \epsilon & \dots & \epsilon \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ \epsilon & \dots & \epsilon & \tau_n & \epsilon \end{pmatrix}$$

and

$$A_1 = \begin{pmatrix} \tau_{\text{in}} & 0 & \epsilon & \dots & \epsilon \\ \epsilon & \epsilon & 0 & \dots & \epsilon \\ \epsilon & \epsilon & \ddots & \ddots & \epsilon \\ \vdots & \ddots & \ddots & \ddots & 0 \\ \epsilon & \dots & \epsilon & \epsilon & \tau_{\text{out}} \end{pmatrix}.$$

Written in component form Eq. (5) gives

$$x_0(k) = \max\{\tau_{\text{in}} + x_0(k-1), x_1(k-1)\},$$

$$x_i(k) = \max\{\tau_i + x_{i-1}(k), x_{i+1}(k-1)\} \quad \text{for } 1 \leq i < n,$$

$$x_n(k) = \max\{\tau_n + x_{n-1}(k), \tau_{\text{out}} + x_n(k-1)\}.$$

If we think of the integer k as labelling the ribosomes, i.e. $x_i(k)$ gives the time at which the k th ribosome leaves the i th codon, then these equations have a simple interpretation. The general term implies that a ribosome will leave codon i either a time τ_i

after it left codon $i-1$, or at the time when the $(k-1)$ th ribosome leaves codon $i+1$ (i.e. time $x_{i+1}(k-1)$), whichever is latest. These alternatives correspond to the interplay between the time of arrival of the appropriate tRNA and the vacation of the next codon by the ribosome ahead of the one in question.

Although the solution of the recursion equation (5) is not straightforward, since there is no inverse to the $\oplus$ operator, by identifying the second term as a vector $\mathbf{b} = A_1 \otimes \mathbf{x}(k-1)$, we can see that this equation is of the form of Eq. (3) and therefore using Theorem 4 its solution is

$$\mathbf{x}(k) = A_0^* \otimes A_1 \otimes \mathbf{x}(k-1).$$

According to the theorem, A_0^* exists since the graph $\mathcal{G}(A_0)$ contains no circuits of positive weight. This can also be seen from a consideration of the first few powers of A_0 (Heidergott et al., 2006). For example, for a system with four transitions ($n=3$)

$$A_0^{\otimes 2} = \begin{pmatrix} \epsilon & \epsilon & \epsilon & \epsilon \\ \epsilon & \epsilon & \epsilon & \epsilon \\ \tau_1 \otimes \tau_2 & \epsilon & \epsilon & \epsilon \\ \epsilon & \tau_2 \otimes \tau_3 & \epsilon & \epsilon \end{pmatrix},$$

$$A_0^{\otimes 3} = \begin{pmatrix} \epsilon & \epsilon & \epsilon & \epsilon \\ \epsilon & \epsilon & \epsilon & \epsilon \\ \epsilon & \epsilon & \epsilon & \epsilon \\ \tau_1 \otimes \tau_2 \otimes \tau_3 & \epsilon & \epsilon & \epsilon \end{pmatrix}.$$

Clearly, the number of non-zero entries decreases as the power increases; indeed, the max-plus sum for the Kleene star contains only a finite number of terms

$$A_0^* = \bigoplus_{k=0}^{\infty} A_0^{\otimes k} = \bigoplus_{k=0}^n A_0^{\otimes k}.$$

We conclude that the Petri net describing translation is governed by the following linear equation:

$$\mathbf{x}(k) = B \otimes \mathbf{x}(k-1), \quad (6)$$

where $B = A_0^* \otimes A_1$ is given by

$$B = \begin{pmatrix} \tau_{\text{in}} & 0 & \epsilon & \epsilon & \cdots & \epsilon \\ \tau_{\text{in}} \otimes \tau_1 & \tau_1 & 0 & \epsilon & \cdots & \epsilon \\ \tau_{\text{in}} \otimes \tau_1 \otimes \tau_2 & \tau_1 \otimes \tau_2 & \tau_2 & 0 & \ddots & \vdots \\ \tau_{\text{in}} \otimes \tau_1 \otimes \tau_2 \otimes \tau_3 & \tau_1 \otimes \tau_2 \otimes \tau_3 & \tau_2 \otimes \tau_3 & \ddots & \ddots & \epsilon \\ \vdots & \vdots & \vdots & \ddots & \tau_{n-1} & 0 \\ \tau_{\text{in}} \otimes \tau_1 \otimes \cdots \otimes \tau_n & \tau_1 \otimes \cdots \otimes \tau_n & \tau_2 \otimes \cdots \otimes \tau_n & \cdots & \tau_{n-1} \otimes \tau_n & \tau_n \oplus \tau_{\text{out}} \end{pmatrix}. \quad (7)$$

The eigenvalue and cyclicity of the matrix B can be found from the corresponding graph $\mathcal{G}(B)$. In Fig. 3 we show $\mathcal{G}(B)$ for the example of $n=3$. We note that the graph is strongly connected, so B is irreducible and has exactly one eigenvalue (Theorem 1). Due to non-zero elements on the diagonal of B , there exist circuits of length one, so the cyclicity of the graph is $\sigma_{\mathcal{G}} = 1$. By inspection of

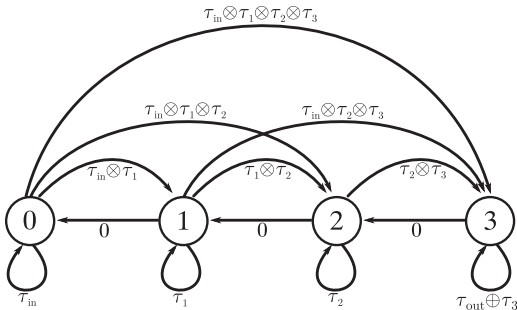


Fig. 3. Diagram showing the weighted, directed graph $\mathcal{G}(B)$ corresponding to the matrix B (Eq. (7)) with $n=3$.

the graph, it is clear that one of the circuits of length one will always have a mean circuit weight $\bar{w}_\gamma$ equal to the maximum mean circuit weight. That is to say, the maximum mean circuit weight, and therefore the eigenvalue of B , will be equal to whichever of the waiting times is largest, i.e.

$$\lambda = \max\{\tau_{\text{in}}, \tau_{\text{out}}, \tau_i \mid 1 \leq i \leq n\}.$$

It follows that the critical graph of B must always contain a circuit of length one and therefore that $\sigma_B = 1$.

Due to the cyclicity theorem (Theorem 3), we conclude that for large k the vector $\mathbf{x}(k)$ is an eigenvector of B . Regardless of the initial condition $\mathbf{x}(0)$, the Petri net describing translation always reaches a steady state described by

$$\mathbf{x}(k) = \lambda \otimes \mathbf{x}(k-1) \quad (8)$$

for $k \geq K_0$, where K_0 is the transient time of B . The component of the eigenvector $x_i(k)$ gives the time at which the i th transition fires for the k th time. The n th component of this vector equation tells us that the transition corresponding to completion of an amino acid chain will fire at time intervals of λ ; thus, the eigenvalue of B can be interpreted as the protein production time interval, P .

As noted above, a way of interpreting the eigenvector is that $x_i(k)$ is the time at which the k th token (ribosome) leaves the i th place (codon); the token enters the i th place at time $x_{i-1}(k)$ and the maximum length of time which a ribosome can stay at a given codon is λ . The proportion of time for which there is a token occupying the i th place in the steady state, i.e. the *occupation density*, is therefore given by

$$\rho_i = \frac{1}{\lambda} (x_i - x_{i-1}), \quad i = 1, \dots, n, \quad (9)$$

where $\{x_i \mid 0 \leq i \leq n\}$ are components of any eigenvector of B , since $x_i(k) - x_{i-1}(k) = x_i(l) - x_{i-1}(l)$ for all $k, l \geq K_0$. We note that the ρ_i are the components of a vector of length n .

It now remains to evaluate the eigenvector. If we define $\mathbf{u} = \mathbf{x}(k-1)$, then from Eq. (8), $\mathbf{x}(k) = \lambda \otimes \mathbf{u}$. We can then use this to evaluate the eigenvector using Eq. (5), which when written as components gives

$$\lambda \otimes u_0 = \tau_{\text{in}} \otimes u_0 \oplus u_1, \quad (10)$$

$$\lambda \otimes u_i = \lambda \otimes \tau_i \otimes u_{i-1} \oplus u_{i+1}, \quad i = 1, \dots, n-1, \quad (11)$$

$$\lambda \otimes u_n = \lambda \otimes \tau_n \otimes u_{n-1} \oplus \tau_{\text{out}} \otimes u_n. \quad (12)$$

Thus, the eigenvector takes a different form depending on the eigenvalue. We identify the following three cases:

4.1. Case (i): $\lambda = \tau_{\text{in}}$

We first consider the case where the parameters are such that the waiting time for initiation of translation is longer than that for tRNA capture and termination, i.e. $\tau_{\text{in}} > \tau_{\text{out}}, \tau_i$ for $1 \leq i \leq n$. We shall call this the *initiation limited regime* because it represents the situation where entry of the ribosomes into the system is the rate limiting process.

Replacing $\lambda \rightarrow \tau_{\text{in}}$ in Eqs. (10)–(12) gives

$$\tau_{\text{in}} \otimes u_0 = \tau_{\text{in}} \otimes u_0 \oplus u_1, \quad (13)$$

$$\tau_{\text{in}} \otimes u_i = \tau_{\text{in}} \otimes \tau_i \otimes u_{i-1} \oplus u_{i+1}, \quad i = 1, \dots, n-1, \quad (14)$$

$$\tau_{\text{in}} \otimes u_n = \tau_{\text{in}} \otimes \tau_n \otimes u_{n-1} \oplus \tau_{\text{out}} \otimes u_n. \quad (15)$$

Using the fact that $\tau_{\text{in}} \neq \tau_{\text{out}}$, it must be the case that $\tau_{\text{in}} \otimes \tau_n \otimes u_{n-1} > \tau_{\text{out}} \otimes u_n$ for Eq. (15) to be consistent. Therefore

$$u_n = \tau_n \otimes u_{n-1}. \quad (16)$$

From Eq. (14), taking $i = n-1$ gives

$$\tau_{in} \otimes u_{n-1} = \tau_{in} \otimes \tau_{n-1} \otimes u_{n-2} \oplus u_n.$$

Since $\tau_{in} \neq \tau_n$, for this to be consistent with Eq. (16), it must be the case that $\tau_{in} \otimes \tau_{n-1} \otimes u_{n-2} > u_n$. Hence

$$\begin{aligned} u_{n-2} &= \tau_{n-1}^{\otimes(-1)} \otimes u_{n-1} \\ &= \tau_{n-1}^{\otimes(-1)} \otimes \tau_n^{\otimes(-1)} \otimes u_n. \end{aligned}$$

Repeated use of Eq. (14) allows us to calculate all the components of the eigenvector in terms of the n th component. The final expression for the eigenvector is then

$$\mathbf{u} = \begin{pmatrix} \tau_1^{\otimes(-1)} \otimes \dots \otimes \tau_n^{\otimes(-1)} \otimes u_n \\ \tau_2^{\otimes(-1)} \otimes \dots \otimes \tau_n^{\otimes(-1)} \otimes u_n \\ \vdots \\ \tau_n^{\otimes(-1)} \otimes u_n \\ u_n \end{pmatrix}.$$

Given this explicit expression for the eigenvector, the occupation density for this regime can be calculated directly using Eq. (9). We find that $\rho_i = \tau_i / \tau_{in}$. Therefore, in the case of the initiation limited regime, the local waiting times directly control the local density.

4.2. Case (ii): $\lambda = \tau_{out}$

In the case where $\tau_{out} > \tau_{in}, \tau_i$ for $1 \leq i \leq n$, the time it takes a ribosome to leave the mRNA is the limiting process, i.e. this is the *termination limited regime*.

The eigenvector for this regime can be calculated using essentially the same method as above. As before, we insert the expression for the eigenvalue $\lambda \rightarrow \tau_{out}$ into Eqs. ((10)–(12)) to obtain

$$\tau_{out} \otimes u_0 = \tau_{in} \otimes u_0 \oplus u_1, \quad (17)$$

$$\tau_{out} \otimes u_i = \tau_{out} \otimes \tau_i \otimes u_{i-1} \oplus u_{i+1}, \quad i = 1, \dots, n-1, \quad (18)$$

$$\tau_{out} \otimes u_n = \tau_{out} \otimes \tau_n \otimes u_{n-1} \oplus \tau_{out} \otimes u_n. \quad (19)$$

In this case, we begin with the first of the three equations. Since $\tau_{in} \neq \tau_{out}$, it must be the case that $u_1 > \tau_{in} \otimes u_0$ and thus

$$u_1 = \tau_{out} \otimes u_0. \quad (20)$$

Using Eq. (18) for $i=1$, this gives

$$\tau_{out} \otimes u_1 = \tau_{out} \otimes \tau_1 \otimes u_0 \oplus u_2.$$

For consistency with Eq. (20), it must be the case that $u_2 > \tau_{out} \otimes \tau_1 \otimes u_0$ and hence

$$\begin{aligned} u_2 &= \tau_{out} \otimes u_1 \\ &= \tau_{out}^{\otimes 2} \otimes u_0. \end{aligned}$$

Repeated use of Eq. (18) gives the other components of the eigenvector

$$\mathbf{u} = \begin{pmatrix} u_0 \\ \tau_{out} \otimes u_0 \\ \tau_{out}^{\otimes 2} \otimes u_0 \\ \vdots \\ \tau_{out}^{\otimes n} \otimes u_0 \end{pmatrix}.$$

In the case of the termination limited regime, the occupation density calculated using this expression is uniform. In fact, $\rho_i = 1$ for all i , the maximum density, indicating that every codon is occupied by a ribosome in this case.

4.3. Case (iii): $\lambda = \max\{\tau_i | 1 \leq i \leq n\}$

The final case is the *tRNA capture limited regime*, where the process is dominated by slow elongation at certain codons. In

other words, the initiation and termination times are both smaller than at least one codon waiting time $\tau = \max\{\tau_i | 1 \leq i \leq n\}$. In this case, the eigenvalue is given by $\lambda = \tau > \tau_{in}, \tau_{out}$. Generally, there will be several positions on the mRNA chain where the slow codon occurs, since there are many more codons in the mRNA chain than there are distinct codon species. For simplicity, we assume initially that there are two places in the bulk of the mRNA, with waiting times equal to τ . That is, there exist integers p and q such that $\tau_p, \tau_q = \tau$, where $1 < p < q < n$.

The calculation of the eigenvector proceeds similar to the previous cases. We begin by substituting for the eigenvalue in Eqs. (10)–(11)

$$\tau \otimes u_0 = \tau_{in} \otimes u_0 \oplus u_1, \quad (21)$$

$$\tau \otimes u_i = \tau \otimes \tau_i \otimes u_{i-1} \oplus u_{i+1}, \quad 1 \leq i < n, \quad (22)$$

$$\tau \otimes u_n = \tau \otimes \tau_n \otimes u_{n-1} \oplus \tau_{out} \otimes u_n. \quad (23)$$

Eq. (21) and the fact that $\tau_{in} \neq \tau$ implies

$$u_1 = \tau \otimes u_0.$$

A similar argument using $\tau_1 \neq \tau$ applied to Eq. (22) with $i=1$ gives

$$\begin{aligned} u_2 &= \tau \otimes u_1 \\ &= \tau^{\otimes 2} \otimes u_0. \end{aligned}$$

We can continue using Eq. (22) in this way until we reach the p th waiting time. Here, the argument fails because $\tau_p = \tau$. Taking $i=p-1$ and then $i=p$ in Eq. (22) we find respectively

$$\tau \otimes u_{p-1} = u_p,$$

$$\tau \otimes u_p = \tau^{\otimes 2} \otimes u_{p-1} \oplus u_{p+1}.$$

Since neither term on the right-hand side of the second equation would contradict the first, all this can tell us is that

$$u_{p+1} \leq \tau^{\otimes 2} \otimes u_{p-1}. \quad (24)$$

Similar logic can be applied if we begin at the other end of the mRNA strand. Consider Eq. (23), since $\tau_{out} \neq \tau$ it follows that

$$u_n = \tau_n \otimes u_{n-1}. \quad (25)$$

Using Eq. (22) with $i=n-1$ gives

$$\tau \otimes u_{n-1} = \tau \otimes \tau_{n-1} \otimes u_{n-2} \oplus u_n.$$

By the now familiar argument, if $\tau_n \neq \tau$, then consistency with Eq. (25) requires that $\tau \otimes \tau_{n-1} \otimes u_{n-2} \geq u_n$ and hence

$$u_{n-1} = \tau_{n-1} \otimes u_{n-2}.$$

We can continue to iterate backwards using Eq. (22) until we reach the q th waiting time $\tau_q = \tau$, where again the argument fails. If we take $i=q$ and then $i=q-1$, we obtain respectively

$$u_q = \tau \otimes u_{q-1},$$

$$\tau \otimes u_{q-1} = \tau \otimes \tau_{q-1} \otimes u_{q-2} \oplus u_q.$$

Again, the second equation can only give the inequality

$$u_q \geq \tau \otimes \tau_{q-1} \otimes u_{q-2}. \quad (26)$$

This approach has led to two non-strict inequalities: Eqs. (24) and (26). These are insufficient to specify the p th and q th components of the eigenvector. However, taking one or the other of these to be the equality, it is possible to continue using Eq. (22) to find a complete and consistent set of components of the eigenvector. In this case, therefore, we find two distinct eigenvectors

(depending on the choice of inequality)

$$\mathbf{u} = \begin{pmatrix} u_0 \\ \tau \otimes u_0 \\ \tau^{\otimes 2} \otimes u_0 \\ \vdots \\ \tau^{\otimes q} \otimes u_0 \\ \tau_{q+1} \otimes \tau^{\otimes q} \otimes u_0 \\ \tau_{q+1} \otimes \tau_{q+2} \otimes \tau^{\otimes q} \otimes u_0 \\ \vdots \\ \tau_{q+1} \otimes \cdots \otimes \tau_n \otimes \tau^{\otimes q} \otimes u_0 \end{pmatrix},$$

where we have assumed that $u_{p+1} = \tau^{\otimes 2} \otimes u_{p-1}$, or

$$\mathbf{u} = \begin{pmatrix} u_0 \\ \tau \otimes u_0 \\ \tau^{\otimes 2} \otimes u_0 \\ \vdots \\ \tau^{\otimes p} \otimes u_0 \\ \tau_{p+1} \otimes \tau^{\otimes p} \otimes u_0 \\ \tau_{p+1} \otimes \tau_{p+2} \otimes \tau^{\otimes p} \otimes u_0 \\ \vdots \\ \tau_{p+1} \otimes \cdots \otimes \tau_n \otimes \tau^{\otimes p} \otimes u_0 \end{pmatrix},$$

where we have assumed that $u_q = \tau \otimes \tau_{q-1} \otimes u_{q-2}$. Any max-plus linear combination of these two vectors is also an eigenvector of B . More generally, if we consider the critical graph of B when there are M non-adjacent codons with waiting times equal to τ , the graph will contain M maximal strongly connected subgraphs and therefore (according to Theorem 2) there are M linearly independent eigenvectors. In particular, note that the critical graphs in cases (i) and (ii) each have only one maximal strongly connected subgraph.

Each of the two eigenvectors above gives rise to a different occupation density profile using Eq. (9)

$$\rho_i = \begin{cases} 1 & \text{for } 1 \leq i \leq q, \\ \tau_i/\tau & \text{for } q < i \leq n \end{cases}$$

and

$$\rho_i = \begin{cases} 1 & \text{for } 1 \leq i \leq p, \\ \tau_i/\tau & \text{for } p < i \leq n. \end{cases}$$

These correspond to queues of ribosomes behind the q th and p th codons, respectively. In mRNAs with more than two isolated codons with waiting times equal to τ , there will be solutions with queues behind each of these “slowest” codons. We will show in the next section that only one of the solutions is realised, and we reserve further discussion until Section 5.2.

5. Simulation results

In this section, we compare the above analytic results with direct numerical simulation of the Petri net shown in Fig. 2(a). The simulations are initialised in a state corresponding to an mRNA with no ribosomes attached. In terms of the Petri net this means that all bottom row places contain a token and all top row places are empty (the first and last places in the chain both contain one token). The simulation evolves from one event (firing of a transition) to the next, updating token positions at each step. The system is allowed to reach a steady state from which the protein production time, P , and the codon occupation density of each place, ρ_i , are measured. These are then compared to the values found from the max-plus algebra: P being the eigenvalue of the matrix B (see Eqs. (6)–(8)), and ρ_i calculated using Eq. (9). The mean density, defined as

$$\rho = \frac{1}{n} \sum_{i=1}^n \rho_i \quad (27)$$

is also calculated.

We first consider a very simple mRNA where all codons are taken to be identical, i.e. they code for the same tRNA and all have the same waiting time. This common waiting time, which will be used as a unit of time, is designated $\bar{\tau}$ so that $\tau_i = \bar{\tau}$ for $1 \leq i \leq n$. We then calculate P , ρ_i and ρ for different values of τ_{in} and τ_{out} .

In Section 5.2 we consider real mRNA sequences from the *S. cerevisiae* genome. The waiting time for a particular tRNA species is assumed to be inversely proportional to the concentration of those molecules found in a typical cell. We estimate this from the gene copy number of each tRNA (Percudani et al., 1997). The mean of these empirical times is taken to be $\bar{\tau}$, which we use as the unit of time.

5.1. Homogeneous mRNAs

Fig. 4 shows colour maps, generated from numerical simulations, for the protein production time and mean density as a function of τ_{in} and τ_{out} for a uniform mRNA of length $n=500$.

Table 1

Details of each regime as given by the max-plus algebra, for a uniform mRNA, where the tRNA capture waiting times are $\tau_i = \bar{\tau}$ for $1 \leq i \leq n$. These are found to match exactly the numerical simulation results.

Quantity	(i) Initiation limited ($\tau_{\text{in}} > \tau_{\text{out}}, \bar{\tau}$)	(ii) Termination limited ($\tau_{\text{out}} > \tau_{\text{in}}, \bar{\tau}$)	(iii) tRNA capture limited ($\bar{\tau} > \tau_{\text{in}}, \tau_{\text{out}}$)
Protein production time P	τ_{in}	τ_{out}	$\bar{\tau}$
Occupation density ρ_i	$\bar{\tau}/\tau_{\text{in}}$	1	1
Mean density ρ	$\bar{\tau}/\tau_{\text{in}}$	1	1

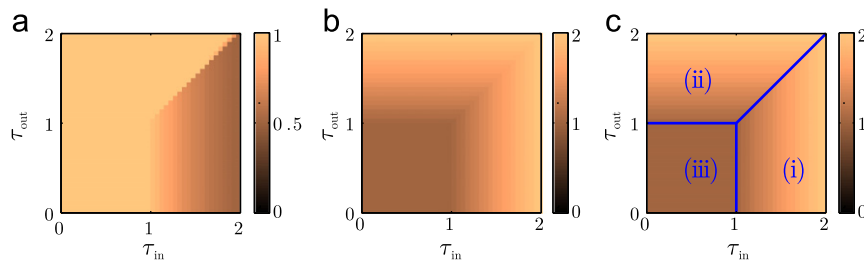


Fig. 4. Colour maps showing how (a) the mean ribosome density, ρ , and (b) protein production time interval, P , vary as a function of τ_{in} and τ_{out} , for a uniform mRNA where all codons have the same tRNA capture time, $\tau_i = \bar{\tau}$. All times are in units of $\bar{\tau}$. Plot (c) shows the results for P again but also includes, in blue, the boundaries between the three regimes, as determined from the max-plus algebra. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

There are three regimes, with the dynamics depending on the relative magnitudes of $\bar{\tau}$, τ_{in} , and τ_{out} . These correspond to the three cases found in the analysis above, i.e. the initiation limited, termination limited and the tRNA capture limited regimes. These results are summarised in Table 1.

Fig. 5 shows how P and ρ vary on sections taken through the phase diagrams shown in Fig. 4. Fig. 5(a) shows ρ as a function of τ_{in} for two fixed values of τ_{out} . Comparison with Fig. 4 shows that when $\tau_{\text{out}} = 0.5\bar{\tau}$ the continuous transition in the behaviour of ρ is expected at $\tau_{\text{in}} = 1$ and conversely when $\tau_{\text{out}} = 1.5\bar{\tau}$ the transition

is expected to be discontinuous. This behaviour is reflected in the sections shown in Fig. 5, where for comparison the results of the max-plus analysis are also plotted. It is clear from this figure that numerical and analytic results agree exactly. Fig. 5(b) makes the same comparisons with the protein production time. In this case, the sections are taken at constant values of τ_{in} .

5.2. Heterogeneous mRNAs

We now consider mRNA sequences from genes YJL136C and YDR382W in *S. cerevisiae*, which we shall denote as mRNA A and mRNA B, respectively. For a given mRNA of length n codons, each of the waiting times $\{\tau_i | 1 \leq i \leq n\}$ is assigned a value chosen from the set of 41 times $\{s_i | 1 \leq i \leq 41\}$ corresponding to each of the 41 tRNA/codon species. We take $\bar{\tau} = (1/41) \sum_{i=1}^{41} s_i$ as the unit of time; from biological experiments this can be estimated as $\bar{\tau} \approx 0.1$ s (Alberts et al., 2008). In Fig. 6 we plot the waiting times at each codon for each of the sequences.

Figs. 7 and 8 show, respectively for each mRNA, simulation results for P and ρ . As was the case with the homogeneous example, we observe three regimes depending on the relative magnitude of τ_{in} , τ_{out} and $\bar{\tau}$, the largest of the τ_i . Again, the simulation results exactly match those from the max-plus algebra, which are summarised in Table 2.

In two of the three regimes the occupation density differs from codon to codon. In Fig. 9 we show the density profile for each mRNA when parameters are chosen such that the system is in the initiation limited phase. We note that for most codons i , $\rho_i < 0.5$; in other models (such as the TASEP model which we discuss in Section 7) the equivalent regime is usually called the *low density phase*. Fig. 10 shows the density profiles for the tRNA capture limited phase. For codons located before the first codon with the longest waiting time ($\tau_p = \tau$), the density $\rho_i = 1$ for $i < p$; the first instance of the slowest codon is causing queueing of ribosomes. It happens that in the case of mRNA B the first slowest codon is at $i=1$, so no queue is observed.

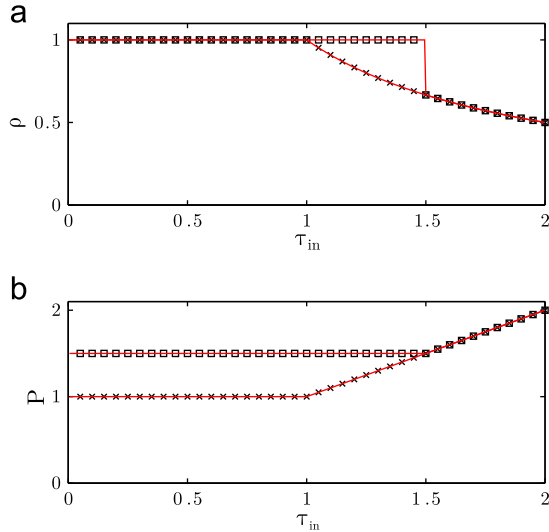


Fig. 5. Comparison of numerical and analytic results for a uniform mRNA. Plots showing (a) the mean density, ρ , and (b) the protein production time, P , at different values of τ_{in} and τ_{out} . Squares show numerical results for $\tau_{\text{out}} = 1.5\bar{\tau}$ and crosses those for $\tau_{\text{out}} = 0.5\bar{\tau}$ at different values of τ_{in} . Red lines show the analytic results from the max-plus algebra. All times are shown in units of $\bar{\tau}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

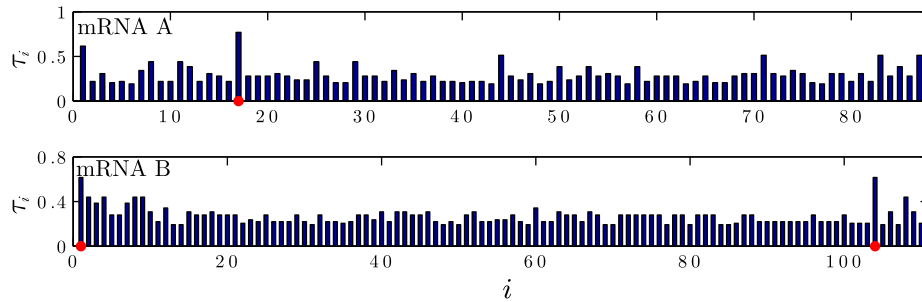


Fig. 6. Waiting times for each codon on mRNA A (top) and mRNA B (bottom). Red dots on the horizontal axis indicate codons which have the largest waiting time: for mRNA A $\tau = 0.7696\bar{\tau}$ and for mRNA B $\tau = 0.6155\bar{\tau}$. Note that each waiting time, τ_i , has a value from the set $\{s_i | 1 \leq i \leq 41\}$. Here, $\bar{\tau} = (1/41) \sum_{i=1}^{41} s_i$ (in general $(1/n) \sum_{i=1}^n \tau_i \neq \bar{\tau}$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

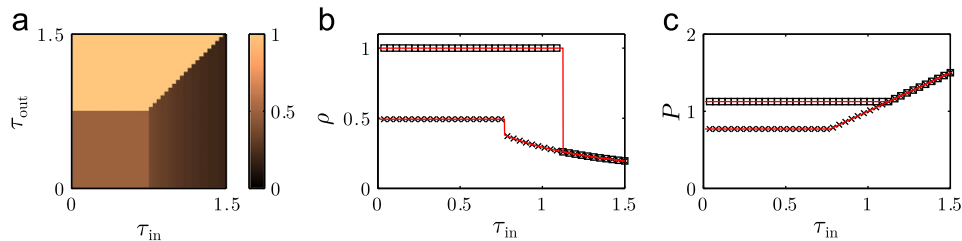


Fig. 7. Comparison of numerical and analytic results for mRNA A showing values of ρ and P for different values of τ_{in} and τ_{out} . In (a) the three regimes are clearly visible. In (b) and (c) squares show numerical results for $\tau_{\text{out}} = 1.125\bar{\tau}$ and crosses for $\tau_{\text{out}} = 0.375\bar{\tau}$. Lines show the analytic results from the max-plus algebra. For this mRNA the maximum internal waiting time is $\tau = 0.7696\bar{\tau}$. All times are in units of $\bar{\tau}$.

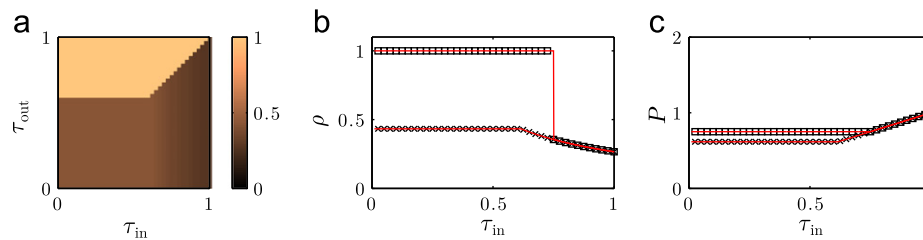


Fig. 8. Comparison of numerical and analytic results for mRNA B showing values of ρ and P for different values of τ_{in} and τ_{out} . In (a) the three regimes are clearly visible. In (b) and (c) squares show numerical results for $\tau_{out} = 0.75\bar{\tau}$ and crosses for $\tau_{out} = 0.25\bar{\tau}$. Lines show the analytic results from the max-plus algebra. For this mRNA the maximum internal waiting time is $\tau = 0.6155\bar{\tau}$. All times are in units of $\bar{\tau}$.

Table 2

Details of each phase as given by the max-plus algebra, for a non-uniform mRNA. Here, τ is the longest internal waiting time ($\tau = \max\{\tau_i | 1 \leq i \leq n\}$) and p is the position of the leftmost codon with waiting time equal to τ .

Quantity	(i) Initiation limited ($\tau_{in} > \tau_{out}, \tau$)	(ii) Termination limited ($\tau_{out} > \tau_{in}, \tau$)	(iii) tRNA capture limited ($\tau > \tau_{in}, \tau_{out}$)
Protein production time P	τ_{in}	τ_{out}	τ
Occupation density ρ_i	τ_i / τ_{in}	1	1 for $1 \leq i \leq p$, τ_i / τ for $p < i \leq n$
Mean density ρ	$\frac{1}{n\tau_{in}} \sum_{i=1}^n \tau_i$	1	$\frac{p}{n} + \frac{1}{n\tau} \sum_{i=p+1}^n \tau_i$

The max-plus analysis of the tRNA capture limited case given in Section 4.3 shows that there are other possible solutions, but only one of these is realised in the simulations. Specifically, instances of the slowest codon appearing further downstream do not give rise to queues, since the rate at which ribosomes arrive at those codons is too low. The cyclicity theorem and Eq. (2) in particular, describe a convergent iteration process, but do not address the question of what happens if there are several eigenvectors that the process can converge to. This can be analysed as a reachability problem with the different possible eigenvectors being reachable from different initial conditions (Butkovic, 2010). In the context of translation the natural initial condition is to have all codons vacant, corresponding to a newly transcribed mRNA. This corresponds to the initial vector $\mathbf{x}(0)$, where $x_0(0) = 0$ and $x_i = \epsilon$ for $i \neq 0$; i.e. we start counting the elapsed time from 0 at the first initiation event. Only the eigenvectors corresponding to a queue behind the first slowest codon are reachable from this initial condition.

In Fig. 6 we see that for mRNA B there are two instances of the slowest codon. In this case, therefore, according to the analysis in Section 4.3, there are two distinct eigenvectors. The second eigenvector – another steady state solution for the density profile of mRNA B – can be reached by manipulating the numerical simulation. We first allow the system to reach steady state and then artificially increase the waiting time on the slow codon at position $i = 104$ by a small amount $\delta\tau$. This means that $i = 104$ is now the unique slowest codon and a queue forms behind it. The system is now allowed to come to a steady state again. If now we reverse the $\delta\tau$ change, the queue behind codon 104 is found to persist (data not shown), showing that this second steady state is indeed an eigenvector of the problem.

6. Introduction of stochasticity

Above we have examined a deterministic version of the model where, rather than choosing random waiting times from a Poisson

distribution, we have used the mean of the distribution. Simulation of a Petri net with waiting times chosen from a distribution is straightforward, its description using max-plus algebra is less so. The problem boils down to a product of irreducible max-plus matrices such that $\mathbf{x}(k+1) = B(k) \otimes \mathbf{x}(k)$ for $k \geq 0$, where $\{B(k) | k \in \mathbb{N}\}$ is an independent and identically distributed (i.i.d.) sequence of random matrices (Heidergott et al., 2006; Mairesse, 1997). It can be shown that for an i.i.d. sequence of matrices, which have fixed support and are irreducible, a max-plus Lyapunov exponent exists and that this is equal to the asymptotic growth rate. In the present model, this is related to the mean protein production time. Unfortunately however, there is currently no method available for calculating this quantity. For this reason, here we will present only a brief discussion of how simulation results for a stochastic Petri net compare with the deterministic case.

We consider a uniform mRNA of length $n = 500$ codons, where waiting times for initiation, termination and tRNA capture are chosen from exponential distributions with means τ_{in} , τ_{out} and $\bar{\tau}$, respectively. We perform simulations where we allow the system to reach steady state before measuring the time averaged protein production time and codon occupation density, which we again denote P and ρ , respectively. As shown in Fig. 11(a) and (d) we again see three regimes depending on which waiting time is the largest (τ_{in} , τ_{out} or $\bar{\tau}$).

There are some significant differences between the stochastic and deterministic models. Fig. 11 shows, that in the stochastic case, the boundary for the tRNA capture limited regime is at $\tau_{in}, \tau_{out} \approx 2\bar{\tau}$, rather than $\tau_{in}, \tau_{out} = \bar{\tau}$, which was found from the deterministic model. In addition, within the tRNA capture limited regime, the density is significantly reduced compared with the deterministic case ($\rho \approx 0.75$ compared to $\rho = 1$) and the protein production time is increased ($P \approx 2.7\bar{\tau}$ compared to $P = \bar{\tau}$). This is as expected since allowing the internal waiting times to vary will lead to gaps between ribosomes. The fact that the difference between the stochastic and deterministic models in this regime is large can be attributed to the fact that the rate limiting process involves choosing waiting times for each of the $n = 500$ places representing the codons. In the other regimes, the effect of the stochasticity is less severe as the limiting process involves only one place. Deep within the initiation or termination limited regimes ρ and P are almost the same as in the deterministic model. The general effect of stochastic waiting times is that both density and protein production time increase compared to the deterministic case. The origin of this effect can be understood by considering ribosomes occupying two consecutive codons, if the waiting time drawn for the leading ribosome is long, it can delay the ribosome behind it. If on the other hand, the time drawn for the leading ribosome is short, it is unlikely to affect the second ribosome since in this case the second ribosome's waiting time will determine its progress. This bias suggests that ribosomes will generally move more slowly along the mRNA. We shall return to

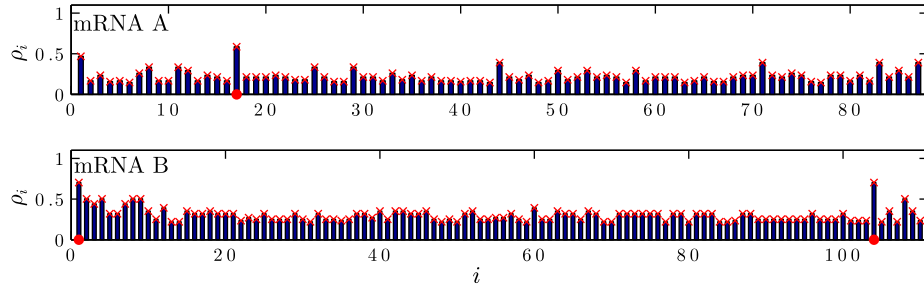


Fig. 9. Occupation density profiles for each mRNA, when parameters are chosen such that the system is in the initiation limited phase. Parameters for mRNA A are $\tau_{in} = 1.3125\bar{\tau}$, $\tau_{out} = 0.0375\bar{\tau}$, $\tau = 0.7969\bar{\tau}$ and for mRNA B $\tau_{in} = 0.875\bar{\tau}$, $\tau_{out} = 0.025\bar{\tau}$, $\tau = 0.6155\bar{\tau}$. Bars show numerical results and crosses show results from the max-plus algebra. Red dots on the horizontal axis indicate the locations of the slowest codons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

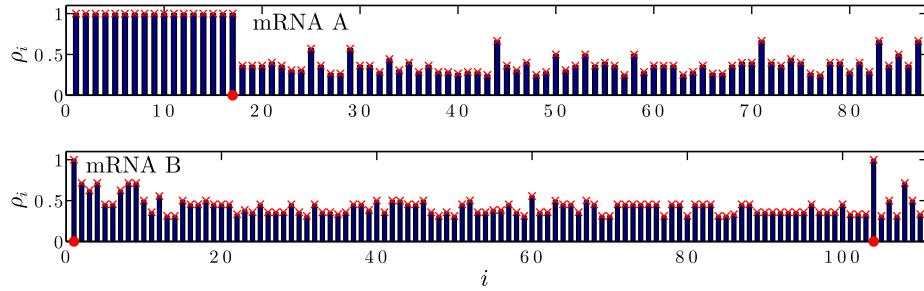


Fig. 10. Occupation density profiles for each mRNA, when parameters are chosen such that the system is in the tRNA capture limited phase. Parameters for mRNA A are $\tau_{in} = 0.0375\bar{\tau}$, $\tau_{out} = 0.0375\bar{\tau}$, $\tau = 0.7969\bar{\tau}$ and for mRNA B $\tau_{in} = 0.025\bar{\tau}$, $\tau_{out} = 0.025\bar{\tau}$, $\tau = 0.6155\bar{\tau}$. Bars show numerical results and crosses show results from the max-plus algebra. Red dots on the horizontal axis indicate the locations of the slowest codons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

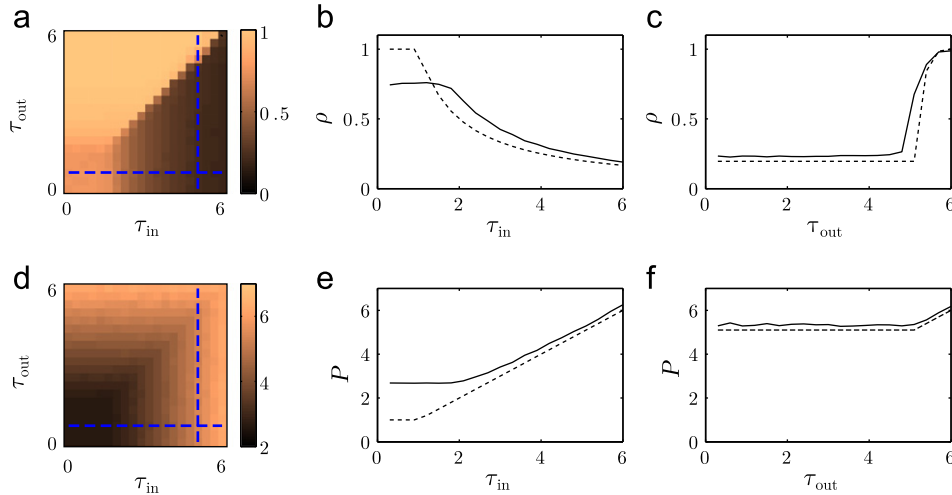


Fig. 11. Simulation results for a stochastic Petri net representing a uniform mRNA of length $n=500$ codons, where each codon corresponds to the same tRNA species. Plots (a) and (d) show colour plots of the mean occupation density and protein production time interval, respectively for different values of τ_{in} and τ_{out} . The other plots show line graphs at cross sections through the colour maps as indicated by the dashed blue lines. Dashed lines in (b), (c), (e) and (f) show results for the corresponding deterministic Petri net. All times are given in units of the mean tRNA capture time $\bar{\tau}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

this point below, where we will give a basic example in which the overall behaviour is determined by the maximum of two exponentially distributed random numbers.

For non-uniform mRNAs, the effect of stochasticity in the initiation and termination limited regimes is similar: both ρ and P are increased compared to the deterministic model. In the tRNA capture limited regime, the situation is more complicated and features such as multiple queues are observed. Discussion of such phenomena is beyond the scope of the present paper. Here, we only briefly consider one consequence of stochasticity which has

played an important role in other translation models (Chou and Lakatos, 2004; Dong et al., 2007).

It has been reported in a widely used alternative model for translation (the TASEP, as discussed in Section 7) that several slow codons in close proximity have a more dramatic effect on the protein production rate from an mRNA than slow codons in isolation. In the deterministic Petri net at steady state, the token release time interval from a place corresponding to a slow codon, which is in isolation, is the same as that from the second of a pair of places corresponding to adjacent identical slow codons. To see

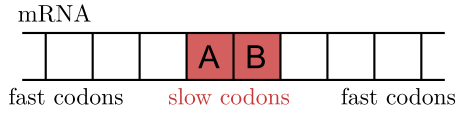


Fig. 12. Colour on-line. Schematic diagram showing a cluster of two slow codons labelled A and B. Ribosomes translate from left to right. All other codons have much shorter waiting times compared to A and B.

this, imagine an mRNA with only two slow codons which appear next to each other, and which we shall denote A and B as depicted in Fig. 12. We know from the steady state analysis of the tRNA capture limited regime given in Section 4.3 that these will cause a queue in which every preceding codon is occupied by a ribosome. In order to maintain the steady state, as soon as a ribosome leaves slow codon B, the ribosome at codon A must move to B and a new ribosome must arrive at A. Therefore, the time interval between ribosomes leaving codon B is not influenced by the presence of the preceding codon.

Although the deterministic Petri net does not predict any effects due to clusters of slow codons we can, using a simple toy mRNA sequence, predict quantitatively how a cluster affects the protein production rate in the stochastic version of the Petri net model. Consider first an mRNA with very fast initiation and termination containing only very fast codons, with the exception of a single slow codon somewhere in the bulk. We focus on the release time interval of tokens from the place corresponding to the slow codon. If the place corresponding to the slow codon has mean waiting time τ_{slow} and we assume that all of the other waiting times are so fast that this place is refilled with a new token immediately after the previous one leaves, then the mean release time interval for tokens from this place is equal to τ_{slow} .

We return to the pair of identical slow codons as shown in Fig. 12, but now consider stochastic waiting times drawn from the (identical) distributions for each slow place $\tau_1^A, \tau_2^A, \dots$, and $\tau_1^B, \tau_2^B, \dots$. We set $t=0$ when the system is in the steady state and a token enters place B (since the other codons are very fast, a token also enters place A at this time). The first token leaves place B at time $t = \tau_1^B$, the next token leaves B at $t = \tau_2^B + \max\{\tau_1^A, \tau_1^B\}$ and the next at $t = \tau_3^B + \max\{\tau_2^A, \tau_2^B\} + \max\{\tau_1^A, \tau_1^B\}$. That is, the i th token leaves place B at

$$t = \tau_i^B + \sum_{j=1}^{i-1} \max\{\tau_j^A, \tau_j^B\}.$$

The i th interval Δ_i between two consecutive tokens leaving B is therefore

$$\Delta_i = \tau_i^B - \tau_{i-1}^B + \max\{\tau_{i-1}^A, \tau_{i-1}^B\}.$$

Taking the average, and noting that the distribution for each slow place is the same, gives

$$\langle \Delta_i \rangle = \langle \max\{\tau_{i-1}^A, \tau_{i-1}^B\} \rangle.$$

Thus, the mean release time interval is the mean of the maximum of two numbers drawn consecutively from the same exponential distribution. This can easily be shown² to be $3\tau_{\text{slow}}/2$, where τ_{slow} is the mean waiting time of the slow codons. This demonstrates that, indeed, clusters of slow codons can have a marked effect in the stochastic Petri net model and supports our earlier assertion that, in general, stochastic waiting times lead to slower movement

of ribosomes along the mRNA. We discuss further implications of introducing stochasticity to the model in the conclusions section.

7. Relationship to other translation models: TASEP

As discussed in the introduction section, an alternative framework for describing mRNA translation, is the TASEP (MacDonald et al., 1968; Derrida et al., 1992; Chou and Lakatos, 2004). In that model, ribosomes are represented by particles which hop in one direction along a 1D lattice of sites, which represent the codons of the mRNA. The current model shows many similarities to the TASEP but the dynamics unfold in an essentially different way.

The dynamics of the TASEP are most often simulated via Monte Carlo methods, using a random-sequential update rule, or using continuous time Monte Carlo (Bortz et al., 1975). In the former case, time is discretised and in each time step, lattice sites are chosen at random with uniform probability. If the chosen site contains a particle and the next site is vacant the particle is advanced with some probability p ; particles are injected from the leftmost site and removed from the rightmost site with probabilities α and β , respectively. For a lattice with n sites this is repeated n times in each time step. In the continuous time version, exactly one particle is moved in each time step but the choice of particle is based on the hopping probabilities and the duration of the time step drawn from the corresponding Poisson distribution. Both methods are equivalent in that they are the realisation of the usual master equation in continuous time. This last point means that multiplying all probabilities by a common factor leads only to a rescaling of time. Under some conditions the random sequential TASEP can be solved exactly (Derrida et al., 1992) and also a mean field treatment which ignores spatial correlations is often used.

The major difference between this and our timed Petri net picture is that in the TASEP only one particle can move at a given instant in time. In the Petri net, as soon as any token move becomes possible, it is executed, which implies that events can happen simultaneously. Biologically, the movement of ribosomes from one codon to the next is not instantaneous, therefore, at any one time there may be many ribosomes moving between codons. In this sense, we suggest that the Petri net more closely models the microscopic dynamics of this system.

Results from the Petri net model can most easily be compared with the TASEP by identifying $\alpha\delta t = 1/\tau_{\text{in}}$, $\beta\delta t = 1/\tau_{\text{out}}$ and, in the case of a uniform mRNA, $p\delta t = 1/\bar{\tau}$, where δt is the time step. Although the uniform TASEP produces a very similar phase diagram to that of Fig. 4, the subtle differences in the dynamics do lead to some important differences in the macroscopic behaviour. The analytic results from the max-plus treatment for the Petri net model and from a mean field approximation for the TASEP are summarised in Table 3. The most obvious difference is that the boundaries of the regimes differ between the two models. Specifically the boundaries for the tRNA capture limited regime (usually called the maximal current phase in the TASEP literature) are at $\tau_{\text{in}}, \tau_{\text{out}} = 2\bar{\tau}$ instead of $\bar{\tau}$ in the deterministic Petri net. In fact, the phase boundaries in the TASEP are the same as those in the stochastic Petri net model discussed in the previous section; which appears to suggest a congruence between the two stochastic models. However, the density and protein production time are different in all three models. In the tRNA capture limited regime we have $\rho = 1/2$ and $P = 4\bar{\tau}$ in the TASEP, compared with $\rho = 1$ and $P = \bar{\tau}$ in the deterministic Petri net and $\rho \approx 3/4$ and $P \approx 2.7\bar{\tau}$ in the stochastic Petri net.

A TASEP with an ordered-sequential update has been studied by some authors (Rajewsky and Schreckenberg, 1997; Rajewsky et al., 1998; Romano et al., 2009) and is particularly applicable to,

² If two times τ_1 and τ_2 are random numbers, where the probability that τ_i has a value x is given by an exponential distribution with mean τ_{slow} , i.e. $P(\tau_i = x) = \tau_{\text{slow}}^{-1} e^{-x/\tau_{\text{slow}}}$, then the probability that $m = \max\{\tau_1, \tau_2\}$ has a value x is given by $P(m = x) = P(\tau_1 = x) \cdot P(\tau_2 < x) + P(\tau_2 = x) \cdot P(\tau_1 < x)$. The mean value of m is then just the expectation value of $P(m = x)$.

Table 3

Comparison of the results from the Petri net and TASEP models for a uniform mRNA. The TASEP results are from a mean field treatment, which has been shown to very closely match random-sequential Monte Carlo simulations for large systems (Derrida et al., 1992).

Regime	Deterministic Petri net			Random-sequential TASEP		
		ρ	P		ρ	P
(i) Initiation limited	$\tau_{in} > \tau_{out}$	$\bar{\tau}/\tau_{in}$	τ_{in}	$\tau_{in} > \tau_{out}$	$\bar{\tau}/\tau_{in}$	$\frac{\tau_{in}^2}{\tau_{in} - \bar{\tau}}$
(ii) Termination limited	$\tau_{in} > \bar{\tau}$	1	τ_{out}	$\tau_{in} > 2\bar{\tau}$	$\bar{\tau}\left(1 - \frac{1}{\tau_{out}}\right)$	$\frac{\tau_{out}^2}{\tau_{out} - \bar{\tau}}$
	$\tau_{out} > \tau_{in}$			$\tau_{out} > \tau_{in}$		
(iii) tRNA capture limited	$\tau_{out} > \bar{\tau}$	1	$\bar{\tau}$	$\tau_{out} > 2\bar{\tau}$	$4\bar{\tau}$	1/2
	$\tau_{out}, \tau_{in} < \bar{\tau}$			$\tau_{in}, \tau_{out} < 2\bar{\tau}$		

for example, traffic flow models, movement of molecular motors, and – as we study here – mRNA translation. This update rule corresponds more closely to the Petri net model. Sites are taken in turn from right to left and updated according to the hopping probabilities. However, this has remained a less favoured update rule since analytic approaches run into several difficulties, particularly in the physical interpretation of hopping probabilities. Unlike the random-sequential case, for the ordered update, time is not continuous, i.e. scaling the probabilities does not lead to a simple scaling of time (for further discussion see Rajewsky et al., 1998). The advantage of the Petri net picture is therefore clear: we can include the fact that multiple events can happen simultaneously, whilst retaining (at least in the deterministic case) an analytically soluble mathematical framework.

Another difference between the current model and the TASEP is in the biological interpretation of the waiting time of the ribosome on each codon. In the random-sequential TASEP, the hopping probability from a site is chosen based on the abundance of tRNAs which corresponds to that codon. A recent study (Ciandrini et al., 2010) shows that this is in fact the wrong interpretation of the hopping probability. Ciandrini et al. (2010) have developed an extension to the TASEP, where ribosomes take two internal states. They identify two times: the waiting time for a ribosome to capture the correct tRNA, $\tau_{capture}$, and the time it takes for the ribosome to physically move from one site to the next, τ_{move} . The former depends on the availability of tRNAs, whilst the latter does not. Moreover, Ciandrini et al. argue that the capture of the tRNA can occur independently of whether or not there is a vacancy to the right of the ribosome. A commonly held misconception in applying the TASEP to translation is that the model represents the limit $\tau_{move} \rightarrow 0$ whereas Ciandrini's model shows that it is the opposite $\tau_{capture} \rightarrow 0$ limit which recovers the original TASEP model. Importantly, the TASEP limit does not describe the biologically relevant regime ($\tau_{move} \ll \tau_{capture}$). In the timed Petri net, the place waiting times correspond to $\tau_{capture}$, hence we explicitly operate in the biologically relevant limit.

8. Concluding remarks

We have presented a new model of ribosome dynamics during mRNA translation. Algebra on the max-plus semi-ring lends itself to the description of systems in which discrete events occur depending on the fulfilment of conditions. This has previously been used to study, for example, distributed software systems and automated manufacturing or industrial control systems. We have shown here that biological systems represent another area where such methods can be applied. The Petri net is a useful tool for visually representing discrete event systems, and the wealth of previous work on describing Petri nets using max-plus has allowed us to develop a framework for predicting the dynamical

behaviour of elongating ribosomes, given only the codon sequence of an mRNA. The present approach offers several advantages over other models, namely that it explicitly operates in the biological limit (see Section 7). The max-plus algebra allows exact results for the deterministic approximation, and the Petri net description allows for stochastic numerical simulation.

The analytic treatment we have presented is applicable to a deterministic version of the model and we have discussed the modifications to the system, when ribosome waiting times are chosen from a distribution. Stochasticity in the translation process has been shown to be one of the most important sources of phenotypic noise, i.e. cell-to-cell variation in protein levels (Ozbudak et al., 2002). By considering the variability of waiting times due to the intrinsic diffusive tRNA movement, this model allows quantitative investigation of the resulting non-Poissonian fluctuations in the protein abundance (Thattai and van Oudenaarden, 2001; Elowitz et al., 1998). For example, Thattai and van Oudenaarden (2001) show that the strength of the noise in protein abundance can be altered independently of the mean by changing the translational efficiency; our model provides a formalism to examine how slow codon arrangement can be used to tune translational efficiency. The present work therefore represents the first step in a new direction for protein synthesis modelling. Whilst numerical simulation of a stochastic Petri net is straightforward, there are currently few methods or algorithms available for the study of sequences of i.i.d. max-plus matrices. Therefore, this work also represents a new source of motivation in this area.

There is also scope for introducing features which bring the model closer to the biology, for example: including a finite time for the physical movement of the ribosomes (Ciandrini et al., 2010); taking into account the fact that ribosomes cover more than one codon at a time (Shaw et al., 2003, 2004); allowing for a finite pool of ribosomes or other resources (Adams et al., 2008; Brackley et al., 2010a,b, 2011). These extensions would raise no additional conceptual issues, they simply make the algebra more complicated.

In summary, we have presented a new model of translation, which uses max-plus algebra to solve a Petri net description of the system. The microscopic dynamics are more realistic than in other translation models and calculation of quantities such as density profiles and protein production rates is straightforward. Unlike other translation models, the max-plus algebra also allows for exact analytic solutions for inhomogeneous mRNA sequences.

Acknowledgements

The authors thank Ian Stansfield and Luca Cinadrini for valuable discussions on the work. D.S.B. acknowledges support from the Engineering and Physical Sciences Research Council

Grants EP/E050441/1 and EP/I016643/1 (CICADA: Centre for Interdisciplinary Computational and Dynamical Analysis and CICADA Cross-disciplinary Feasibility Account). C.A.B., M.C.R. and M.T. acknowledge support from the Biotechnology and Biological Sciences Research Council Grants BB/F00513/X1 and BB/G010722, and the Scottish Universities Life Science Alliance (SULSA).

References

- Adams, D.A., Schmittmann, B., Zia, R.K.P., 2008. Far-from-equilibrium transport with constrained resources. *J. Stat. Mech.*, P06009.
- Alberts, B., Johnson, A., Walter, P., Lewis, J., 2008. *Molecular Biology of the Cell*, 5th edn. Garland Pub Inc.
- Bacelli, F., Cohen, G., Olsder, G.J., Quadrat, J.P., 1992. *Synchronization and Linearity: An Algebra for Discrete Event Systems*. Wiley.
- Bortz, A.B., Kalos, M.H., Lebowitz, J.L., 1975. A new algorithm for monte carlo simulation of Ising spin systems. *J. Comput. Phys.* 17, 10–18.
- Brackley, C.A., Romano, M.C., Grebogi, C., Thiel, M., 2010a. Limited resources in a driven diffusion process. *Phys. Rev. Lett.* 105, 078102.
- Brackley, C.A., Romano, M.C., Thiel, M., 2010b. Slow sites in an exclusion process with limited resources. *Phys. Rev. E* 82, 051920.
- Brackley, C.A., Romano, M.C., Thiel, M., 2011. The dynamics of supply and demand in mRNA translation. *PLoS Comput. Biol.* 7, e1002203.
- Brockmann, R., Beyer, A., Heinisch, J.J., Wilhelm, T., 2007. Posttranscriptional expression regulation: what determines translation rates. *PLoS Comput. Biol.* 3, e57.
- Butkovic, P., 2010. *Max-Linear Systems: Theory and Algorithms*, 1st edn. Springer.
- Chou, T., Lakatos, G., 2004. Clustered bottlenecks in mRNA translation and protein synthesis. *Phys. Rev. Lett.* 93, 198101.
- Ciandrini, L., Stansfield, I., Romano, M.C., 2010. Role of the particle's stepping cycle in an asymmetric exclusion process: a model of mRNA translation. *Phys. Rev. E* 81, 051904.
- Cohen, G., Dubois, D., Quadrat, J., Viot, M., 1985. A linear-system-theoretic view of discrete-event processes and its use for performance evaluation in manufacturing. *IEEE Trans. Automat. Control* 30, 210–220.
- Cohen, G., Gaubert, S., Quadrat, J.P., 1999. Max-plus algebra and system theory: where we are and where to go now. *Ann. Rev. Control* 23, 207–219.
- Derrida, B., Domany, E., Mukamel, D., 1992. An exact solution of a one-dimensional asymmetric exclusion model with open boundaries. *J. Stat. Phys.* 69, 667–687.
- Dong, J.J., Schmittmann, B., Zia, R.K.P., 2007. Inhomogeneous exclusion processes with extended objects: the effect of defect locations. *Phys. Rev. E* 76, 051113.
- Elf, J., Berg, O.G., Ehrenberg, M., 2001. Comparison of repressor and transcriptional attenuator systems for control of amino acid biosynthesis operons. *J. Mol. Biol.* 313, 941–954.
- Elf, J., Ehrenberg, M., 2005. Near-critical behavior of aminoacyl-tRNA pools in *E. coli* at rate-limiting supply of amino acids. *Biophys. J.* 88, 132–146.
- Elowitz, M.B., Levine, A.J., Siggia, E.D., Swain, P.S., 1998. Stochastic gene expression in a single cell. *Science* 297, 1183–1186.
- Gaubert, S., Mairesse, J., 1999. Modeling and analysis of timed Petri nets using heaps of pieces. *IEEE Trans. Automat. Control* 44, 683–697.
- Gilchrist, M.A., Wagner, A., 2006. A model of protein translation including codon bias, nonsense errors, and ribosome recycling. *J. Theor. Biol.* 239, 417–434.
- Heidergott, B., Olsder, G.J., van der Woude, J., 2006. *Max Plus at Work: Modeling and Analysis of Synchronized Systems: A Course on Max-Plus Algebra and Its Applications*. Princeton University Press.
- MacDonald, C.T., Gibbs, J.H., Pipkin, A.C., 1968. Kinetics of biopolymerization on nucleic acid templates. *Biopolymers* 6, 1–25.
- Mairesse, J., 1997. Products of irreducible random matrices in the $(\max, +)$ algebra. *Adv. Appl. Prob.* 29, 444–447.
- Murata, T., 1989. Petri nets: properties, analysis and applications. *Proc. IEEE* 77, 541–580.
- Ozbudak, E.M., Thattai, M., Kurtser, I., Grossman, A.D., van Oudenaarden, A., 2002. Regulation of noise in the expression of a single gene. *Nature Genetics* 31, 69–73.
- Percudani, R., Pavesi, A., Ottonello, S., 1997. Transfer RNA gene redundancy and translational selection in *Saccharomyces cerevisiae*. *J. Mol. Biol.* 268, 322–330.
- Petri, C.A., 1962. *Kommunikation Mit Automaten*. Ph.D. Thesis. Institut für Instrumentelle Mathematik, Bonn.
- Rajewsky, N., Santen, L., Schadschneider, A., Schreckenberg, M., 1998. The asymmetric exclusion process: comparison of update procedures. *J. Stat. Phys.* 92, 151–194.
- Rajewsky, N., Schreckenberg, M., 1997. Exact results for one-dimensional cellular automata with different types of updates. *Physica A* 245, 139–144.
- Robinson, M., Lilley, R., Little, S., Emtage, J., Yarranton, G., Stephens, P., Millican, A., Eaton, M., Humphreys, G., 1984. Codon usage can affect efficiency of translation of genes in *Escherichia coli*. *Nucl. Acids Res.* 12, 6663–6671.
- Romano, M.C., Thiel, M., Stansfield, I., Grebogi, C., 2009. Queueing phase transition: theory of translation. *Phys. Rev. Lett.* 102, 198104.
- Shaw, L.B., Kolomeisky, A.B., Lee, K.H., 2004. Local inhomogeneity in asymmetric simple exclusion processes with extended objects. *J. Phys. A* 37, 2105.
- Shaw, L.B., Zia, R.K.P., Lee, K.H., 2003. Totally asymmetric exclusion process with extended objects: a model for protein synthesis. *Phys. Rev. E* 68, 021910.
- Sørensen, M.A., Kurland, C.G., Pedersen, S., 1989. Codon usage determines translation rate in *Escherichia coli*. *J. Mol. Biol.* 207, 365–377.
- Thattai, M., van Oudenaarden, A., 2001. Intrinsic noise in gene regulatory networks. *Proc. Nat. Acad. Sci.* 98, 8614–8619.