

Luminescence Control in the Marine Bacterium *Vibrio fischeri*: An Analysis of the Dynamics of *lux* Regulation

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A mathematical model has been developed based on the fundamental properties of the control system formed by the *lux* genes and their products in *Vibrio fischeri*. The model clearly demonstrates how the components of this system work together to create two, stable metabolic states corresponding to the expression of the luminescent and non-luminescent phenotypes. It is demonstrated how the cell can “switch” between these steady states due to changes in parameters describing metabolic processes and the extracellular concentration of the signal molecule *N*-3-oxohexanoyl-L-homoserine lactone. In addition, it is shown how these parameters influence how sensitive the switch mechanism is to cellular LuxR and *N*-3-oxohexanoyl-L-homoserine lactone and complex concentration. While these properties could lead to the collective phenomenon known as quorum sensing, the model also predicts that under certain metabolic circumstances, basal expression of the *lux* genes could cause a cell to luminesce in the absence of extracellular signal molecule. Finally, the model developed in this study provides a basis for analysing the impact of other levels of control upon *lux* regulation.

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Introduction

The LuxR-LuxI family of transcriptional regulators is used by a wide variety of bacteria to regulate the expression of certain genes in response to population density, in a process termed autoinduction (Sitnikov *et al.*, 1995). Much detailed knowledge is now available about the components of this regulatory system in the marine bacterium *Vibrio fischeri*, and there is now a need to focus on the exact mechanism by which these components might work together to form an efficient sensory system. Mathematical modelling allows the system to be viewed as a whole to gain insight into how the expression of the *lux* genes of *V. fischeri* allow it to produce and respond to a class of signal molecules known as acylated homoserine lactones (AHLs).

Abbreviations used: AHLs, acylated homoserine lactones; OHHL, *N*-3-oxohexanoyl-L-homoserine lactone; CRP, cAMP receptor protein; OHL, *N*-octanoyl-L-homoserine lactone.

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AHLs were first identified in luminous species of *Vibrio* which live both as symbionts in the light organs of some marine fish and squid and as free-living organisms (Ruby & McFall-Ngai, 1992). In the free-living state these bacteria are non-luminescent; however, they become highly luminescent when at high cell density and enclosed within the host light organ (Sitnikov *et al.*, 1995). Several bacterial species contain *lux*-type transcriptional regulators that control the production of virulence factors (Hugouvieux-Cotte-Pattat *et al.*, 1996). Phenotypes such as swarming (Eberl *et al.*, 1995; Allison & Hughes, 1991) and plasmid conjugation (Piper *et al.*, 1993) have also been shown to involve *lux*-type control. The *lux* regulon of *V. fischeri* is organised into two operons (the left and right) which are transcribed divergently (Shadel & Baldwin, 1991). The *luxR* gene on the left operon (O_L) encodes a transcriptional activator which, together with autoinducer, binds to a 20 bp inverted repeat between the *lux* operons (termed the *lux* box) to activate the transcription of both operons. Although there is some debate as to whether or not the autoinducer binds directly to

the LuxR protein to form a complex (Sitnikov *et al.*, 1995), it is now generally accepted that it does. It is also debated, but likely, that LuxR binds to the *lux* box as a dimer or trimer, in a process triggered by the binding of autoinducer to monomeric LuxR (Choi & Greenberg, 1992). The right operon (O_R) contains *luxI*, the gene encoding a synthase for the autoinducer *N*-3-oxohexanoyl-L-homoserine lactone (OHHL), and the genes *luxCDABEG* which encode the luminescence enzymes (Schaefer *et al.*, 1996).

The classic view of autoinduction involves the gradual build-up of autoinducer within a growing population of cells that produce it at a basal rate, where the OHHL is capable of diffusing into and out of the cell membranes (Fuqua *et al.*, 1996). In this way, the OHHL concentration within the cells reflects the size of the population as a whole. In *V. fischeri*, the binding of extra OHHL with cellular LuxR produces more complex which further stimulates the *lux* genes, resulting in high levels of bioluminescence. This scenario, termed "quorum sensing" is clearly the case for growing cultures within enclosed spaces such as a light organ or culture flask; however, many studies indicate a more diverse role for *lux* control in bacteria. The build-up of autoinducer is subject to more factors than simply population density; growth rate, permeability of the cell membrane, shape and degree of enclosure of the culture can all potentially affect autoinducer concentration. There is increasing evidence that autoinducer signalling is a major factor in the ecology of mixed bacterial populations such as biofilms (Davies *et al.*, 1998). In such populations, *lux* type signalling may be complicated by the variety of autoinducers and the potential for signalling and interference between bacterial species and higher organisms (Fuqua & Greenberg, 1998; Givskov *et al.*, 1996).

Other levels of control have been identified which may influence the dynamics of *lux* control in *V. fischeri* (Figure 1). Catabolite repression effects the expression of *lux* genes cloned in *Escherichia coli* through cAMP receptor protein (CRP) (Dunlap & Greenberg, 1985) and other possible control mechanisms include the heat-shock-induced chaperone protein GroESL (Adar & Ulitzur, 1993) and the LexA protein (Sitnikov *et al.*, 1995). Two other autoinducers have been found in *V. fischeri*, one which is synthesised by LuxI (but in much lower quantities than OHHL), and another which is produced by a separate gene designated *ainS* (Kuo *et al.*, 1994). Indirect data suggest that the latter autoinducer, *N*-octanoyl-L-homoserine lactone (OHL), competitively inhibits the association of OHHL with LuxR resulting in a complex with a markedly lower *lux* operon-inducing specific activity (Kuo *et al.*, 1996). Substrate availability, transcription and translation rates could also influence the luminescence phenotype.

The present work uses modelling techniques to describe how the organisation of the *lux* genes in *V. fischeri* forms a regulatory mechanism, where

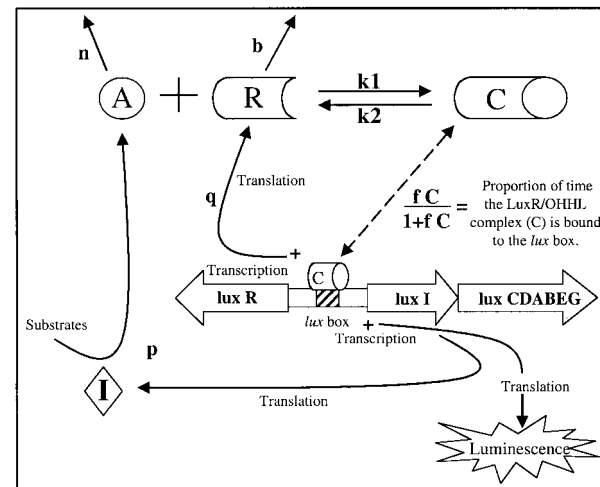


Figure 1. Model for the dynamics between the central components of *lux* regulation in *Vibrio fischeri*. The binding reaction between OHHL (A) and LuxR (R) to form a complex (C) is described using the rate constants k_1 and k_2 for the binding and dissociation reactions, respectively. The diffusion of A through the cell membrane is determined by the diffusion constant n , and R is broken down according to the constant b . The proportion of time *lux* box is occupied by complex is described by the expression $fC/1+fC$. The rate at which A and R molecules are produced while the complex is bound to the *lux* box is symbolised by p and q , respectively. The activity of LuxI (I) in synthesising A is included within the constant p , assuming that the availability of substrates is not a limiting factor.

the level of gene induction is responsive to the concentration of extracellular signal molecule. The study focuses upon the *lux* regulatory system within a single *V. fischeri* cell, first in an OHHL-free environment where the OHHL diffusing out of the cell is lost from the system, and second in the presence of extracellular OHHL. The model is used to predict how the metabolic properties of the cell influence the sensitivity of its *lux* system to changes in cellular LuxR and OHHL and the significance of these results is discussed for a variety of biological contexts.

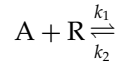
Theory

Development of a model

The model of *lux* control in *V. fischeri* is described in the simplest possible form, including only those components seen as being central to the dynamics of the system. This allows the behaviour of the model under different biological situations to be represented and compared by changing the relevant key parameters. The analysis is carried out from the perspective of *lux* gene regulation within a single cell. A comparison is made between an isolated, free-living cell without OHHL in its external environment and a cell exposed to exter-

nal OHHL, such as would be expected in the presence of other cells, artificially added OHHL or in a confined space.

The overall structure of the model system developed to describe *lux* regulation in *V. fischeri* is shown diagrammatically in Figure 1. It is now generally accepted that OHHL autoinducer binds directly to the LuxR protein to form a complex (Sitnikov *et al.*, 1995), and this is represented in the model by the interaction between OHHL (A) and LuxR protein (R) to form an active complex (C). The dynamics of this reaction is described by the binding rate constant k_1 and dissociation rate constant k_2 :



Thus, the binding rate is proportional to the product of the concentrations of A and R, whereas the rate of the dissociation reaction is proportional to the concentration of C only:

$$\text{Binding reaction rate} = k_1AR \quad (1a)$$

$$\text{Dissociation reaction rate} = k_2C \quad (1b)$$

where A, R and C are the concentrations of A, R and C.

The proportion of time during which the *lux* box is occupied by the LuxR/OHHL complex (C) is described as a function of the concentration of complex (C) within the cell. When the concentration of C is high the *lux* box will be occupied almost all the time. When the concentration is low we can reasonably assume the fraction of time that the site is occupied to depend linearly on the concentration of C, say fC where C is the concentration of C. Requiring this fraction to tend to unity for high concentrations leads to a functional form as follows.

Proportion of time *lux* box is occupied by complex :

$$= \frac{fC}{1+fC} \quad (2)$$

Binding of the LuxR/OHHL complex (C) to the *lux* box has a positive effect upon the expression of both the left and right *lux* operons of *V. fischeri* (Shadel & Baldwin, 1992; Sitnikov *et al.*, 1995). The rates of production of A and R molecules from the right and left *lux* operons while the *lux* box is occupied, is represented by the constants p and q , respectively (Figure 1). Thus:

$$\text{LuxR synthesis rate} = q \times \frac{fC}{1+fC} \quad (3a)$$

$$\text{OHHL synthesis rate} = p \times \frac{fC}{1+fC} \quad (3b)$$

Although *luxR* and *luxI* are known to have a basal rate of expression while the *lux* box is unoccupied (Dunlap & Kuo, 1992), this factor is not included in the model. This is because the focus of this study is to describe the dynamics of *lux* gene regulation which result from their up-regulation and positive feedback. It is worth noting, however, that cellular levels of OHHL and LuxR would never actually fall to zero in the real-life system.

Because of its lactone ring, the OHHL molecule would be expected to be very stable at the pH range of a living cell (Morrison & Boyd, 1992). For this reason, the major source of loss of OHHL (A) from the cell is assumed to be *via* diffusion out through the cell membrane. The net rate of diffusion out of the cell is assumed to be proportional to the cellular concentration of A, and proceeds at a rate determined by the diffusion constant (n) such that:

$$\text{Diffusion rate of OHHL} = nA \quad (4)$$

The value of n results from the properties of the *V. fischeri* cell membrane such as permeability and surface area.

Loss of the LuxR protein is assumed to occur through enzymatic breakdown. The rate of the degradation reaction is proportional to its cellular concentration and to proceed at a rate determined by the constant b such that:

$$\text{Degradation rate of LuxR} = bR \quad (5)$$

Many models in bacterial systems assume that the cells are in a state of growth and accordingly assume that each component of the system is undergoing dilution; however, this is not necessarily the case in the present model. Dilution terms are usually compounded within the loss terms (in this case n and b), and the only real difference in a mathematical sense is that it introduces a lower limit to the values of n and b . The LuxR-OHHL complex is not described as undergoing dilution and is assumed to be lost *via* dissociation into its constituent parts. The parameters used in this model are summarised in Table 1.

The present model represents a single, isolated bacterium in which OHHL is only able to diffuse out of the cell. Using the definitions described above, the interactions between core components of the *V. fischeri lux* system can be represented symbolically by three coupled differential equations. These describe the rate of change in the concentrations of OHHL (dA/dt) and LuxR (dR/dt) inside a single bacterium:

$$\frac{dA}{dt} = k_2C - k_1AR - nA + p \frac{fC}{1+fC} \quad (6a)$$

$$\frac{dR}{dt} = k_2C - k_1AR - bR + q \frac{fC}{1+fC} \quad (6b)$$

Table 1. Definitions of parameters

Description		
A	Cellular concentration of autoinducer OHHL	$m\ l^{-3}$
A_{ex}	Extracellular concentration of autoinducer OHHL	$m\ l^{-3}$
A_2	Cellular concentration of autoinducer OHL	$m\ l^{-3}$
R	Cellular concentration of Lux R	$m\ l^{-3}$
C	Cellular concentration of LuxR/OHHL complex	$m\ l^{-3}$
n	Diffusion constant of OHHL through the cell membrane	t^{-1}
b	Degradation constant for LuxR	t^{-1}
p	Formation of OHHL due to <i>lux</i> gene activity	$m\ l^{-3}\ t^{-1}$
q	Formation of LuxR due to <i>lux</i> gene activity	$m\ l^{-3}\ t^{-1}$
k_1	Rate constant of binding reaction between OHHL and LuxR	$l^3\ m^{-1}\ t^{-1}$
k_2	Rate constant of dissociation reaction of OHHL and LuxR	t^{-1}
f	-	$l^3\ m^{-1}$

$$\frac{dC}{dt} = k_1AR - k_2C \quad (6c)$$

In order to make a comparison between a cell with and without OHHL in its external environment, the basic model is modified to incorporate the existence of in-diffusion. This change is represented by modifying the differential equation describing the rate of change in the concentration of OHHL (equation (6a)), by the addition of the variable A_{ex} , which represents the concentration of extracellular OHHL. Thus, equation (6a), which now includes a term for the in-diffusion of OHHL, becomes:

$$\frac{dA}{dt} = k_2C - k_1AR - n(A - A_{ex}) + p \frac{fC}{1+fC} \quad (6d)$$

Steady state conditions and stability analysis

One of the first requirements for a model describing the function of the *lux* control circuit in *V. fischeri* is that it must be capable of maintaining a steady state. Assuming that a major role of the *lux* circuit is to act as a level of control upon the expression of the luminescence phenotype, its dynamics must allow for the maintenance of a state of unchanging concentrations of the central components OHHL (A), LuxR (R) and complex (C). For a cell in steady state, the rate of change in these components is zero, so possible steady state values of A, R and C are obtained by solving $dA/dt = 0$, $dR/dt = 0$ and $dC/dt = 0$ simultaneously with respect to A, R and C. Thus, in order to determine the presence of steady states in the model, equations (6b-d) are set equal to zero and solved simultaneously for A, R and C (Appendix I). Solutions for an isolated cell, in the absence of

external OHHL, are obtained by setting $A_{ex} = 0$ in this result.

The ability of a system to exist in steady state has little biological meaning unless that state exhibits some degree of local stability, as small fluctuations in conditions are inevitable in biological systems. The stability of each steady state derived from the present model has been analysed by the standard procedure of examining the Jacobian of the three-equation system (equation (6b-d)) (Appendix II).

Results

Luminescence in the absence of extracellular OHHL

Regulation of the *lux* genes within a *V. fischeri* cell without OHHL in its external environment is described by the system of three differential equations (equations (6a-c)). This system has been analysed to determine its basic properties in terms of the presence of steady states, the stability of those states and the influence of the parameter values. Three possible steady states exist for this system. The first steady-state (S_0) is stable and is always present regardless of the values of the parameters k_1 , k_2 , p , q , n , b and f (Figure 2). The concentrations of R and C at this steady-state are equal to zero and the concentration of A is equal to the external concentration of OHHL (A_{ex}) which, in the case of an isolated cell is also equal to zero. This steady state represents a cell in a non-induced state of luminescence, and a cell in this state would contain only the levels of A, R and C that result from basal expression of the *lux* genes.

In Appendix I it is shown that the existence of the two non-zero steady states, S_1 and S_2 , depends on the inequality:

$$k_1pqf > 4k_2nb \quad (7)$$

Furthermore, the steady states S_1 and S_2 both have positive C and thence positive A and R and are thus biologically relevant. When inequality (7) is not satisfied, the cell is only capable of the steady state S_0 . Such a cell will always return to non-induced levels of A and R production, no matter what the starting levels of LuxR and OHHL might be, and is thus unable to sustain a luminescent response. When the parameters k_1 , k_2 , p , q , n , b and f are such that inequality (7) is satisfied, the cell is capable of reaching the steady states S_1 and S_2 . Figure 2 shows the relative concentrations of A, R and C at the steady states S_0 , S_1 and S_2 , and it can be seen from this Figure that S_1 always has A, R and C values which lie between those of S_0 and S_2 .

Because the ability of a system to exist in steady state has little biological meaning unless that state exhibits some degree of local stability, the steady states S_1 and S_2 have been analysed for local stability. As described in Appendix II, it can be shown that the non-zero steady states are stable if they

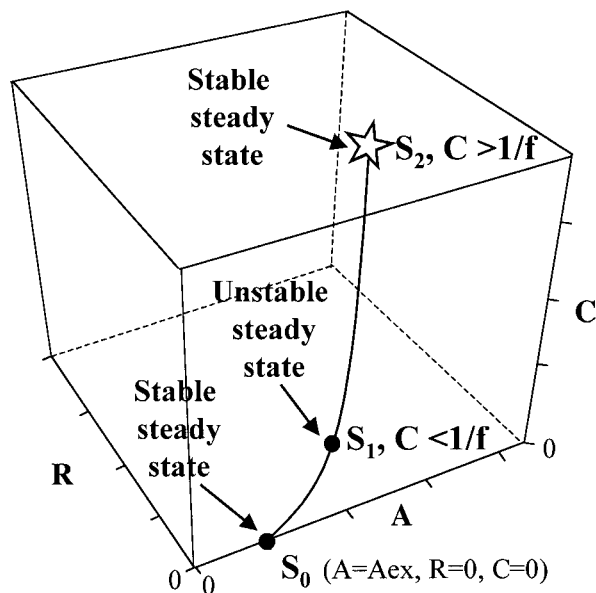


Figure 2. Relative concentrations of OHHL (A), LuxR (R) and complex (C) at each of the three steady states: S_0 , S_1 and S_2 . The system of three differential equations (6b-d) describing the dynamics of *lux* regulation in *V. fischeri*, has three possible steady states. S_0 is stable and is always present. S_1 and S_2 exist provided $k_1 p q f > 4 k_2 n b$ (inequality (7)). Furthermore, because the concentration of C must be above the threshold value of $1/f$ for the steady states to be stable (Appendix I), S_1 is always unstable and S_2 is always stable.

satisfy the inequality:

$$C > \frac{1}{f} \quad (8)$$

This means that there is a threshold concentration of cellular LuxR-OHHL complex necessary for the cell to be capable of sustaining a stable equilibrium. Furthermore, the steady state S_2 has $C > 1/f$, and the steady state S_1 has $C < 1/f$ (Appendix II). Thus, S_2 is always stable and S_1 is always unstable (Figure 2).

These results indicate that a single *V. fischeri* cell, without OHHL in its external environment, is capable of reaching the stable steady state (S_2) with heightened levels of LuxR, OHHL and complex and correspondingly higher luminescence. The ability of the cell to reach this state is dependent upon the parameter values as shown in inequality (7). This inequality shows that the parameters can be classed into two groups: those which, when increased sufficiently, result in the non-zero steady states (k_1 , p , q and f) and those which have the opposite effect (k_2 , n and b). These two groups correspond to the effects the parameters have upon the cells' ability to form the LuxR-OHHL complex. The rate at which OHHL and LuxR are produced upon *lux* gene activation (represented by p and q ,

respectively) and the efficiency with which they form a complex and bind to the *lux* box (represented by k_1/k_2 and f) are all parameters which increase the concentration of cellular complex. The rates at which OHHL and LuxR are removed from the system (n and b , respectively) decrease cellular complex.

Figure 3 indicates the behaviour of the system around the stable steady state, S_2 . The position of the steady state is shown on a 3D-plot of the cellular concentrations of OHHL (A) versus LuxR (R) versus complex (C), and a sphere is drawn with S_2 at its centre. The stability of S_2 is demonstrated by the fact that from any starting point on the surface of the sphere, the system defined by the model will eventually end up at S_2 . The shading on the surface of the sphere represents the time the system takes to reach S_2 from that starting point. This pattern of behaviour remains the same for a large range of radii; the sphere shown includes concentrations of R below one-third of the steady-state concentration. These results indicate that under the circumstances outlined above, the biological system would reach and sustain the state of elevated *lux* gene expression represented by S_2 .

Significance of the unstable steady state

According to the present model, when the parameter values are such that they satisfy inequality (7), there are two non-zero steady states, S_1 and S_2 . In the stability analysis in Appendix II, it is shown that S_2 is always a stable steady state and S_1 is always unstable. The difference between the stable steady state at S_2 and the unstable steady state at S_1 is shown in Figure 4. As in the last Figure, a sphere is drawn with the steady state at its centre and all the points on the surface of that sphere are taken as starting concentrations for A, R and C. This time, the shading of the surface differentiates where the system ends up over time from those different starting points: dark shading indicates rapid convergence to the stable steady state S_0 and light shading indicates slower convergence to the stable steady state S_2 .

In the biological system, the steady state S_1 would never be sustained due to its instability. However, it does influence the properties of the A, R, C co-ordinate system such that very small differences in the concentrations of these three parameters can determine whether or not the system ends up at the non-induced steady state S_0 , or the luminescent state S_2 (Figure 4). These dynamics form an explanation for the "switch-like" behaviour of the *lux* system, where the cell reaches a point where it suddenly "turns on" the luminescence. Also, because S_1 can be relatively close to S_2 (Figure 2), the small amounts of A, R and C present in the cell due to basal gene expression could potentially cause the cell to spontaneously luminesce. This would tend to happen at high k_1 , p , q or f and low k_2 , n or b .

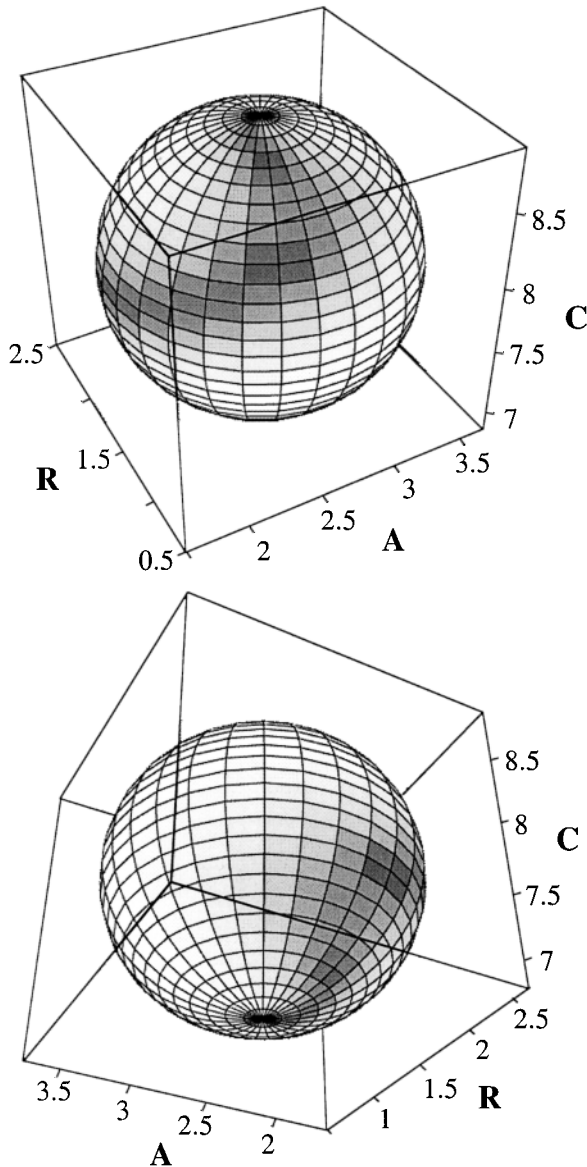


Figure 3. An example of the behaviour of the model system around the stable steady state, S_2 . This steady state is positioned at the centre of a sphere drawn on a 3D-plot of the cellular concentrations of OHHL (A) versus LuxR (R) versus complex (C), where the upper and lower graphs depict opposite sides of the sphere. The stability of this steady state is demonstrated by the fact that from any starting point on the surface of the sphere, the system defined by the model will eventually end up at S_2 . The shading on the surface of the sphere represents the relative time it takes for the system to reach S_2 from that starting point, with black being zero, and white being 15 seconds or longer. The parameter values used for this example are $k_1 = 20$ ($l^3 m^{-1} t^{-1}$), $k_2 = 10$ (t^{-1}), $n = 10$ (t^{-1}), $b = 3$ (t^{-1}), $p = 30$ ($m l^{-3} t^{-1}$), $q = 5$ ($m l^{-3} t^{-1}$) and $f = 1$ ($l^3 m^{-1}$).

The influence of extracellular OHHL

The regulation of the *lux* genes within a *V. fischeri* cell in the presence of extracellular

OHHL has been examined by adding an extra term describing the diffusion of OHHL into the cell to the basic model, as shown in equation (6d). The concentration of external OHHL is represented in the model by the parameter A_{ex} . Appendix II shows that the presence of extracellular OHHL does not greatly affect the stability of the steady states. The unstable steady state S_1 with $C < 1/f$ may cease to exist, and the condition for stability of the steady state S_2 with $C > 1/f$ becomes:

$$C > \frac{11 + nA_{ex}/p}{f(1 - nA_{ex}/p)} \quad (9)$$

which holds unless A_{ex} becomes large. Figure 5 shows how A_{ex} influences the existence of steady states when other parameters of the system (k_1 , k_2 , p , q , n , b and f) are fixed. For example, given parameter values corresponding to the point represented by a star in Figure 5, and in the absence of extracellular OHHL, no non-zero steady state is possible. However, by increasing A_{ex} the region that admits steady states can be increased until it encompasses the star symbol. Thus, a *V. fischeri* cell with parameter values such that it is non-luminescent in the absence of A_{ex} , will, upon exposure to an increasing concentration of external OHHL, remain non-luminescent until it exceeds a threshold value after which the cell becomes capable of luminescence. Such a cell will remain luminescent until the concentration of external OHHL drops again (Figure 5). Thus, a *V. fischeri* cell in a low OHHL environment, for example in a small group or within a thin biofilm, can behave according to the dynamics described above, where there is a sudden switch between the luminescent and non-luminescent phenotype. Such cells can be extremely sensitive to small relative changes in their internal OHHL and LuxR concentrations.

Discussion

This study focuses upon the *lux* regulatory system within a single *V. fischeri* cell. The system is at first examined for a cell in an OHHL-free environment where the OHHL diffusing out of the cell is lost from the system. The model is then extended to consider the way in which such a cell might respond to OHHL in its surroundings, without making any assumptions about the source of external OHHL (A_{ex}). This way of considering *lux* control can be applied to a variety of biological contexts. For example, the system without A_{ex} would apply to a single, isolated cell, or a thin biofilm of cells in an environment where the OHHL they produce is quickly washed away from the system. The model with A_{ex} would apply to any circumstances resulting in the accumulation of OHHL around the cell due to the enclosure of the system or its presence within a large dense, population of cells.

The present study demonstrates that a simple representation of the balance between the pro-

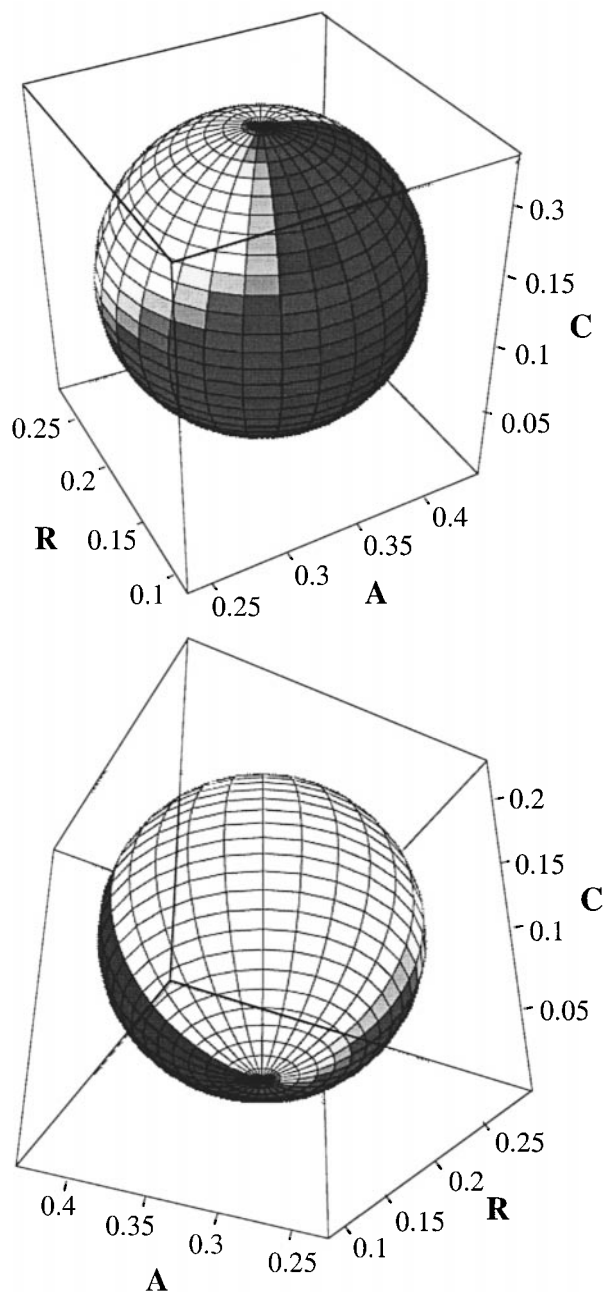


Figure 4. An example of the behaviour of the model system around the unstable steady state, S_1 . The steady state is positioned at the centre of a sphere drawn on a 3D-plot of the cellular concentrations of OHHL (A) versus LuxR (R) versus complex (C), where the upper and lower graphs depict opposite sides of the sphere. The instability of this steady state is demonstrated by the fact that from any starting point on the surface of the sphere, the system defined by the model will eventually end up at either S_0 or S_2 . The shading on the surface of the sphere represents the relative time it takes for the system to reach either S_0 or S_2 from that starting point, with black being zero, and white being 15 seconds or longer. Points on the sphere surface facing towards S_0 converged rapidly to this steady state, whereas the others converged more slowly to the luminescent steady state, S_2 . The parameter values used for this example are $k_1 = 20$ ($\text{l}^3 \text{m}^{-1} \text{t}^{-1}$), $k_2 = 10$ (t^{-1}), $n = 10$ (t^{-1}), $b = 3$ (t^{-1}), $p = 30$ ($\text{m} \text{l}^{-3} \text{t}^{-1}$), $q = 5$ ($\text{m} \text{l}^{-3} \text{t}^{-1}$) and $f = 1$ ($\text{l}^3 \text{m}^{-1}$).

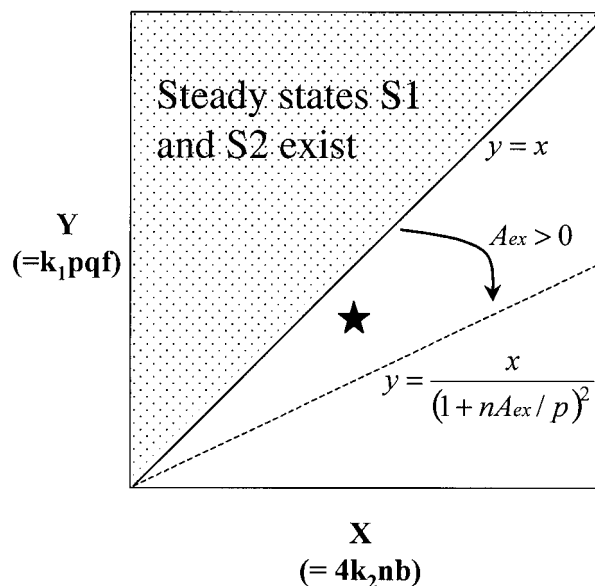


Figure 5. The effect of external OHHL (A_{ex}) upon the existence of the steady states: S_1 and S_2 . The area above the line $y=x$, represents the parameter space which fulfils inequality (7) ($k_1pqf > 4k_2nb$), and thus represents the parameter conditions under which the system can support the luminescent steady state, S_2 . This parameter space expands to the dotted line when A_{ex} is increased, so that any cells with parameter values which placed them above this line, which were previously unable to luminesce (represented by the star symbol), become capable of luminescence upon exposure to external OHHL.

duction and loss of three molecules, namely OHHL (A), LuxR (R) and complex (C), each dependent upon the others for its production, can potentially form the structure of a sophisticated control system. The *lux* regulatory system is represented by three core attributes: the binding reaction between A and R to form the LuxR-OHHL complex (C), positive feedback upon A and R production correlated to cellular C concentration, and the ability of A to diffuse through the cell membrane (Figure 1). These qualities alone produce a system with all the basic qualities attributed to the *lux* regulatory system of *V. fischeri*. The model system has two stable steady states, designated S_0 and S_2 , which correspond to a non-luminescent cell, and an induced luminescent cell, respectively (Figure 2). A cell in the steady state S_0 would actually be producing a small amount of A and R because there is some basal expression of the *lux* genes when the *lux* box is unoccupied (Kuo *et al.*, 1996).

Because of the dynamics of the unstable steady state S_1 (Figure 4), the model predicts that the cell can switch suddenly between the stable steady states S_0 and S_2 at a threshold concentration of cellular A, R or C. This behaviour is typically

observed in laboratory batch cultures of *V. fischeri* (Sitnikov *et al.*, 1995). Finally, because of the ability of extracellular OHHL (A_{ex}) to diffuse into the cell, its concentration can act as a signal controlling the luminescent phenotype, such that an increasing concentration of A_{ex} will reach a threshold value after the cell will switch to the induced steady state S_2 (Figure 5). Thus, a growing population of non-induced *V. fischeri* cells within an enclosed space, each producing OHHL at the basal rate, would be expected to increase the concentration of A_{ex} until it reached a threshold level at which all the cells would begin to luminesce. Such behaviour corresponds to the classical description of quorum sensing (Fuqua *et al.*, 1996).

The model system predicts other properties of *lux* regulation in *V. fischeri*, some of which could explain the mechanisms behind experimental observations. First, a single, isolated cell, or a cell under conditions of very low external OHHL would still be capable of reaching the luminescent steady state if there were changes in properties linked to the parameter values shown in inequality (7). For example, a decrease in cell membrane permeability would have the effect of lowering the parameter n (equation (4)), pushing the cell into a state where it is able to reach steady state S_2 (Figure 2). A decrease in the degradation rate of LuxR (b), an increase in the efficiency with which *luxR* or *luxI* are transcribed and translated (q or p) or an increase in the efficiency of the binding reaction (k_1/k_2 and f) could also cause such a cell to luminesce. Such changes would also effect the A , R , and C co-ordinates of the unstable steady state S_1 (Figure 2), altering the threshold concentrations of A and R at which the switch to the luminescent phenotype would take place (Figure 4). If the values of k_1 , k_2 , p , q , n , b and f were such that the unstable steady-state S_1 was close to S_0 , the basal expression of the non-induced *lux* genes could be sufficient for the cell to spontaneously switch to the luminescent phenotype.

This degree of sensitivity would not be advantageous in situations where luminescence is only desirable at high population densities. However, the ability of a cell to adjust the sensitivity of its *lux* system could be very important in explaining the behaviour of *lux*-like regulated cells in thin biofilms (Davies *et al.*, 1998). According to the model, even a non-enclosed population of *V. fischeri* cells, under conditions where the OHHL they produce is quickly washed away, could contain cells expressing the luminescent phenotype. The presence of induced cells within a population of non-induced cells could be an important aspect of the ecology of *lux*-like regulation in naturally occurring biofilms. One such example could be the findings of McLean *et al.* (1997), who found that cells from natural biofilms expressed homoserine lactones.

The model system described here, was purposely kept simple in order to identify those basic properties of *lux* regulation in *V. fischeri* which are essen-

tial to the behaviour of the biological system. The model system clearly demonstrates that the basic properties of the *lux* system included in the model are sufficient in themselves to form a regulatory system responsive to extracellular OHHL. The model also provides insight into how specific changes in the properties of the cell, such as membrane permeability, LuxR degradation and the efficiency of *lux* gene transcription and translation, effect the sensitivity of its *lux* system. It should be mentioned, however, that there are several factors not included in the model that could potentially influence the properties of *lux* regulation in *V. fischeri*. For example, one such factor is an additional negative feedback mechanism in which an extremely high concentration of complex inhibits *luxR* transcription (Sitnikov *et al.*, 1995; Shadel & Baldwin, 1992). This was left out of the model as there are indications that it occurs only when the cellular LuxR concentration is extremely high, possibly higher than would naturally occur in *V. fischeri* (Sitnikov *et al.*, 1995).

The present model forms a basis from which the effects of other control mechanisms upon *lux* regulation could be analysed and compared. The ability to respond to several factors, not just OHHL concentration, is a logical requirement for the control of a phenotype that is only beneficial in certain circumstances such as symbiosis. Other bacteria with *lux* control mechanisms clearly respond to nutrient deprivation and/or host factors in addition to their own population density (Latifi *et al.*, 1996). For this reason, the influence of the *lux* regulatory circuit upon bioluminescence in *V. fischeri* must be looked at through the veil of other levels of control with which it may interact. Behaviour predicted by the present model may, in the living cell, be overridden by control mechanisms designed to allow it to respond to other information about its metabolic state and environment which are relevant to the value of expressing the luminescence phenotype.

Future studies in the modelling of *lux* regulation could focus on cyclic AMP receptor protein and its effect upon the stimulation of the left and right *lux* operons, both in the presence and absence of LuxR-OHHL complex (Sitnikov *et al.*, 1995). Understanding the effects of this process is necessary for a full understanding of *lux* control. Similarly, the effects of GroESL, LexA and the production of other signal molecules with the same cell (Kuo *et al.*, 1994), could also be considered in the context of the dynamics of the system, as could the potential impact of the active transport of signal molecules through the cell membrane. Finally, the *lux*-like control systems in different bacterial species could be modelled and compared to that of *V. fischeri*, to analyse any differences in the way that the structure of their *lux* systems enable them to respond to signal molecules.

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Appendix I

To obtain the steady-state concentrations, in the presence of external OHHL, set the time derivatives in equations (6b-d) to zero to get these conditions:

$$k_1AR = k_2C \quad (A1)$$

$$n(A - A_{ex}) = \frac{pfC}{1 + fC} \quad (A2)$$

$$bR = \frac{qfC}{1 + fC} \quad (A3)$$

Substitute equations (A2) and (A3) into equation (A1) to get an equation in C only:

$$k_1 \left[\frac{pfC}{n(1 + fC)} + A_{ex} \right] \left[\frac{qfC}{b(1 + fC)} \right] = k_2C$$

so either $C = 0$ for a null steady state or:

$$k_2nb(fC)^2 + (2k_2nb - k_1pqf - k_1nqfA_{ex})(fC) - (k_2nb + k_1nqfA_{ex}) = 0$$

Define new variables

$$1 + \varepsilon = \frac{k_1 p q f}{4k_2 n b} \quad \text{and} \quad \tau = \frac{n A_{\text{ex}}}{p} \quad (\text{A4})$$

in terms of which the two solutions to this quadratic equation are:

$$fC = 1 + 2\varepsilon + 2(1 + \varepsilon)\tau \pm 2(1 + \varepsilon)\sqrt{\frac{\varepsilon}{1 + \varepsilon} + 2\tau + \tau^2} \quad (\text{A5})$$

Non-zero steady states exist when the argument of the square root is positive:

$$\frac{\varepsilon}{1 + \varepsilon} + 2\tau + \tau^2 > 0$$

is equivalent to:

$$\frac{k_1 p q f}{4k_2 n b} > \left(\frac{1}{1 + \tau}\right)^2$$

and Figure 5 illustrates this relationship using axes $x = 4k_2 n b$ and $y = k_1 p q f$. When there is no external OHHL, $\tau = 0$ and this condition is simply $\varepsilon > 0$ or equivalently $k_1 p q f > 4k_2 n b$ as quoted in equation (7).

Appendix II will require more information about the steady-state concentrations, so take $\varepsilon > 0$ and consider the inequality:

$$\begin{aligned} \frac{\varepsilon}{1 + \varepsilon} + 2\tau + \tau^2 &> \left(\frac{\varepsilon}{1 + \varepsilon}\right)^2 + \frac{2\varepsilon\tau}{1 + \varepsilon} + \tau^2 \\ &= \left(\frac{\varepsilon}{1 + \varepsilon} + \tau\right)^2 \end{aligned}$$

and substitute in equation (A5) to show that:

$$\begin{aligned} fC_+ &> 1 + 2\varepsilon + 2(1 + \varepsilon)\tau + 2(1 + \varepsilon)\left(\frac{\varepsilon}{1 + \varepsilon} + \tau\right) \\ &= 1 + 4\varepsilon + 4(1 + \varepsilon)\tau \end{aligned} \quad (\text{A6})$$

$$fC_- < 1 + 2\varepsilon + 2(1 + \varepsilon)\tau - 2(1 + \varepsilon)\left(\frac{\varepsilon}{1 + \varepsilon} + \tau\right) = 1 \quad (\text{A7})$$

Label the three steady states as:

S_0 when $C = 0$, the null state,

S_1 when $C = C_- < \frac{1}{f}$, and

S_2 when $C = C_+ > \frac{1}{f}$.

Equations (A2) and (A3) imply that A and R are positive when C is positive, so S_2 is always a chemically significant state. Using $1/2 + \varepsilon > g t \sqrt{\varepsilon(1 + \varepsilon)}$ it can be shown that $C_- > 0$ when there is no external

OHHL; otherwise S_1 may have negative concentrations and therefore be of no interest.

Appendix II

Steady states have observable consequences only when they are stable; that is, when the chemical system reacts to small variations in A , R , and C by returning them to their steady-state values. We can investigate stability by using the Jacobian matrix to summarise the behaviour of the time derivatives in equations (6b-d) in the vicinity of a steady state. The Jacobian matrix for our system is:

$$\begin{aligned} J &= \begin{pmatrix} \frac{\partial}{\partial A} \frac{dA}{dt} & \frac{\partial}{\partial R} \frac{dA}{dt} & \frac{\partial}{\partial C} \frac{dA}{dt} \\ \frac{\partial}{\partial A} \frac{dR}{dt} & \frac{\partial}{\partial R} \frac{dR}{dt} & \frac{\partial}{\partial C} \frac{dR}{dt} \\ \frac{\partial}{\partial A} \frac{dC}{dt} & \frac{\partial}{\partial R} \frac{dC}{dt} & \frac{\partial}{\partial C} \frac{dC}{dt} \end{pmatrix} \\ &= \begin{pmatrix} -k_1 R - n & -k_1 A & k_2 + \frac{p f}{(1 + fC)^2} \\ -k_1 R & -k_1 A - b & k_2 + \frac{q f}{(1 + fC)^2} \\ k_1 R & k_1 A & -k_2 \end{pmatrix} \end{aligned}$$

and the characteristic polynomial defined by $\det(\lambda I - J) = 0$ with unit matrix I is:

$$\lambda^3 - c_2 \lambda^2 + c_1 \lambda - c_0 = 0 \quad (\text{A8})$$

where the coefficients are:

$$c_2 = -k_1(A + R) - k_2 - (n + b) \quad (\text{A9})$$

$$\begin{aligned} c_1 &= k_1(nA + bR) + k_2(n + b) + nb \\ &\quad - \frac{k_1 f}{(1 + fC)^2}(qA + pR) \end{aligned} \quad (\text{A10})$$

$$c_0 = \frac{k_1 f}{(1 + fC)^2}(nqA + bpR) - k_2 n b \quad (\text{A11})$$

The importance of the characteristic equation is that its roots $\lambda_1, \lambda_2, \lambda_3$ appear in the functions $\exp(\lambda_i t)$ for $i = 1, 2, 3$ that describe the reaction of the system to small variations in A , R , and C from their steady-state values. Only when all roots are negative will the variations decay with time, indicating a stable steady state.

The Jacobian is independent of A_{ex} and so is unchanged by external OHHL. To simplify this presentation, however, we will now set $A_{\text{ex}} = 0$ to allow these identities to be derived from the steady-state conditions in equations (A1-3):

$$nqA + bpR = \frac{2pqfC}{1 + fC} \quad (\text{A12})$$

$$nbAR = \frac{pqf^2C^2}{(1+fC)^2} \text{ giving } nb(1+fC)^2 = \frac{k_1}{k_2}pqf^2C \quad (\text{A13})$$

$$(n+b)AR = \frac{fC}{1+fC}(qA+pR) \quad (\text{A14})$$

Manipulate (A10) and (A11) using these identities to obtain:

$$c_1 = k_1(nA+bR) + nb + \frac{k_1f^2C}{(1+fC)^2}(qA+pR) \quad (\text{A15})$$

$$c_0 = \frac{k_1pqf^2C(1-fC)}{(1+fC)^3} \quad (\text{A16})$$

Because all variables are positive in the chemical model, the signs of the coefficients can easily be seen in equations (A9), (A15), and (A16). Write the characteristic equation as $(\lambda - \lambda_1)(\lambda - \lambda_2)(\lambda - \lambda_3) = 0$ and expand to show that the coefficients may be expressed as combinations of the roots:

$$\lambda^3 - (\lambda_1 + \lambda_2 + \lambda_3)\lambda^2 + (\lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1)\lambda - (\lambda_1\lambda_2\lambda_3) = 0$$

therefore:

$$c_2 = \lambda_1 + \lambda_2 + \lambda_3 < 0 \text{ always,}$$

$$c_1 = \lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1 > 0 \text{ always, and}$$

$$c_0 = \lambda_1\lambda_2\lambda_3 < 0 \text{ provided that } C > \frac{1}{f}$$

A brief argument shows that all roots, when real, are negative when $C > 1/f$. If $\lambda_1\lambda_2\lambda_3 < 0$ then either all roots are negative or only one is negative. Suppose the latter and say $\lambda_1 < 0$. Now $\lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1 > 0 \Rightarrow (-\lambda_1)(\lambda_2 + \lambda_3) < \lambda_2\lambda_3$ and $\lambda_1 + \lambda_2 + \lambda_3 < 0 \Rightarrow \lambda_2 + \lambda_3 < (-\lambda_1)$ giving $(\lambda_2 + \lambda_3)^2 < \lambda_2\lambda_3 \Rightarrow \lambda_2^2 + \lambda_3^2 < -\lambda_2\lambda_3$ which is impossible when $\lambda_2 > 0$ and $\lambda_3 > 0$. Therefore our supposition is wrong and all three roots are negative.

The characteristic equation may have complex roots occurring in complex-conjugate pairs. These correspond to oscillation about the steady state. Suppose that λ_1 is real, $\lambda_2 = \alpha + i\beta$, and $\lambda_3 = \alpha - i\beta$ where α and β are real. For a stable steady state we now require $\lambda_1 < 0$ and $\alpha < 0$. Using

$\lambda_2 + \lambda_3 = 2\alpha$ and $\lambda_2\lambda_3 = \alpha^2 + \beta^2$ the coefficients can be written as:

$$c_2 = \lambda_1 + 2\alpha$$

$$c_1 = 2\alpha\lambda_1 + \alpha^2 + \beta^2$$

$$c_0 = \lambda_1(\alpha^2 + \beta^2).$$

The third equation immediately gives $\lambda_1 < 0$ when $C > 1/f$. Eliminate λ_1 and β to get the cubic equation:

$$\alpha^3 - c_2\alpha^2 + \frac{1}{4}(c_2^2 + c_1)\alpha - \frac{1}{8}(c_1c_2 - c_0) = 0 \quad (\text{A17})$$

Because $c_2 < 0$ and $c_2^2 + c_1 > 0$, the argument used for the characteristic equation (A8) implies that all possible solutions for α are negative if $c_1c_2 < c_0$. Use equations (A9) and (A15) and the steady-state condition (A1) to write:

$$\begin{aligned} (-c_2)c_1 &= [\text{positive terms} + k_2][\text{positive terms} + nb] \\ &> k_2nb = \frac{k_1}{C}nbAR \end{aligned}$$

and use the steady-state identity (A13) and equation (A16) to write:

$$\begin{aligned} (-c_2)c_1 &> \frac{k_1}{C} \frac{pqf^2C^2}{(1+fC)^2} = \frac{k_1pqf^2C(1+fC)}{(1+fC)^3} \\ &> \frac{k_1pqf^2C(fC-1)}{(1+fC)^3} = -c_0 \end{aligned}$$

Thus $c_1c_2 < c_0$ and α is negative.

We have shown that all roots of the characteristic equation, or their real parts if complex, are certainly negative when $C > 1/f$. Thus, the steady state S_2 is stable. If $A_{\text{ex}} \neq 0$ the steady-state identities (A12-14) are not so brief but the results in this Appendix are unchanged except for a more general condition for stability:

$$fC > \frac{1+\tau}{1-\tau} \quad \text{where} \quad \tau = \frac{nA_{\text{ex}}}{p} \quad (\text{A18})$$

Equation (A6) shows that fC_+ at steady state S_2 is significantly larger than one, so S_2 is stable as long as the concentration of external OHHL is not too large.

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