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DOI:
10.1039/B812094C
10.1039/b812094c

Citation for published version (Harvard):
Welham, P & Stekel, D 2009, 'Mathematical model of the Lux luminescence system in the terrestrial bacterium Photorhabdus luminescens', *Molecular BioSystems*, vol. 5, no. 1, pp. 68-76. https://doi.org/10.1039/B812094C, https://doi.org/10.1039/b812094c

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Mathematical model of the Lux luminescence system in the terrestrial bacterium *Photorhabdus luminescens*†

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Received 15th July 2008, Accepted 25th September 2008
First published as an Advance Article on the web 4th November 2008
DOI: 10.1039/b812094c

A mathematical model of the Lux luminescence system, governed by the operon *luxCDABE* in the terrestrial bacterium *Photorhabdus luminescens*, was constructed using a set of coupled ordinary differential equations. This model will have value in the interpretation of Lux data when used as a reporter in time-course gene expression experiments. The system was tested on time series and stationary data from published papers and the model is in good agreement with the published data. Metabolic control analysis demonstrates that control of the system lies mainly with the aldehyde recycling pathway (LuxE and LuxC). The rate at which light is produced in the steady state model shows a low sensitivity to changes in kinetic parameter values to those measured in other species of luminescent bacteria, demonstrating the robustness of the Lux system.

Published on 04 November 2008 on http://pubs.rsc.org | doi:10.1039/B812094C
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Introduction

Bioluminescent species are widely distributed in nature. It seems that the light-emitting systems employed by the different phylogenetic groups evolved independently—the reactions and structures of the enzymes (luciferases) and substrates (luciferins) involved are very varied. The only common feature is that oxygen is required for the bioluminescent reaction.[1]

Species of luminescent bacteria are found in terrestrial, freshwater and marine environments. Most species belong to the *Aliivibrio*, *Photobacterium* or *Vibrio* genera from the *Vibrionaceae* family (*Gammaproteobacteria*).[2] The genes which encode the enzymes for the luminescent reactions in prokaryotes are known as *lux* genes. *Lux* genes are vertically inherited in the majority of these species, but those of *Shewanella hanedai* and *Shewanella woodyi*, two members of another *Gammaproteobacteria* family, are closely related to those of *Aliivibrio*, suggesting that horizontal gene transfer has occurred in these cases.

Marine species of luminescent bacteria include *Vibrio fischeri*, *Vibrio harveyi* and *Photobacterium phosphoreum*.[3,4] The only light-emitting terrestrial bacterium found so far is *Photorhabdus luminescens*. It was once thought that there was also another terrestrial species, *Xenorhabdus luminescens*, but since 1996 it has been recognised that these are the same species.[5] *Lux* genes have been cloned and sequenced for three strains of *P. luminescens*, and the luciferases produced from these genes have been characterized.[6–9] The luciferase from *P. luminescens* has a very high thermal stability (a half life of over three hours at 45 °C), making the *lux* operon of this organism a very good choice as a reporter system.[7] It was for this reason that *P. luminescens* was chosen for study.

The operon responsible for the reactions involved in terrestrial bacterial luminescence is *luxCDABE*[6] (Fig. 1). *LuxA* and *luxB* encode for the α and β subunits of the luciferase. There is 30% identity between *luxA* and *luxB*, indicating gene

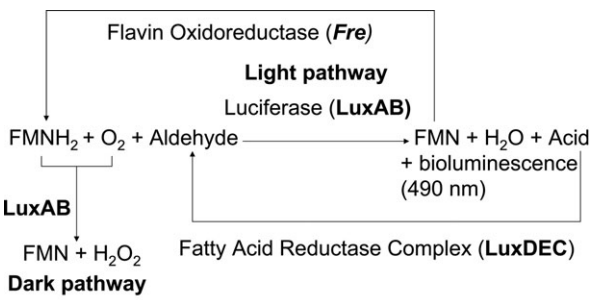

Fig. 1 Reactions catalysed by the products of the LuxABCDE operon in *Photorhabdus luminescens*. In the light pathway the luciferase LuxAB catalyses the oxidation of reduced flavin and an aldehyde to flavin and a long chain fatty acid, resulting in emission of blue-green light. The acid is recycled to aldehyde by the action of the fatty acid reductase complex LuxCDE. Oxidised flavin is reduced by the enzyme Fre. If aldehyde concentration is low or zero the dark pathway is followed, leading to the production of flavin and hydrogen peroxide.

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† Electronic supplementary information (ESI) available: Supplementary tables. See DOI: 10.1039/b812094c

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duplication. It has been suggested that the duplication may have arisen prior to the divergence of the lines leading to present-day luminescent bacteria.[10] The active site is thought to be on the α subunit. The LuxAB enzyme catalyses the oxidation of $FMNH_2$ (reduced flavin) and a long chain fatty aldehyde to oxidised flavin (FMN) and a long chain fatty acid respectively. This reaction results in the emission of blue-green light (wavelength 490 nm).

There are two pathways for the luciferase reaction:

The light pathway, in the presence of aldehyde, leads to production of light by the following sequence of steps:

$$FMNH_2 + LuxAB \rightleftharpoons LuxAB{\cdot}FMNH_2$$

$$LuxAB{\cdot}FMNH_2 + O_2 \rightarrow LuxAB{\cdot}FMNH_2{\cdot}O_2$$

$$LuxAB{\cdot}FMNH_2{\cdot}O_2 + RCHO \rightarrow LuxAB{\cdot}FMNH_2{\cdot}O_2{-}RCHO$$

$$LuxAB{\cdot}FMNH_2{\cdot}O_2{-}RCHO \rightarrow LuxAB + FMN + RCOOH + H_2O + light$$

The dark pathway, in which aldehyde is not consumed, and light is not produced, consists of a sequence of three steps:

$$FMNH_2 + LuxAB \rightleftharpoons LuxAB{\cdot}FMNH_2$$

$$LuxAB{\cdot}FMNH_2 + O_2 \rightarrow LuxAB{\cdot}FMNH_2.O_2$$

$$LuxAB{\cdot}FMNH_2{\cdot}O_2 \rightarrow LuxAB + FMN + H_2O_2$$

It can be seen that light is produced in the light pathway when the $LuxAB{\cdot}FMNH_2O_2{-}RCHO$ complex breaks down to FMN, RCOOH and H_2O. Energy may also be released, however, by the breakdown of the previous intermediate, $LuxAB{\cdot}FMNH_2{\cdot}O_2$, to FMN and H_2O_2 *via* the dark pathway. Light production does not occur in this pathway. The total decay rate (k_T) is given by:

$$k_T = (k_L A + k_D K_A)/(K_A + A)$$

where k_L and k_D are the decay rates for the light and dark pathways respectively, A is the aldehyde concentration, and K_A is the dissociation constant for the aldehyde.[11]

The *luxCDE* genes encode the fatty acid reductase complex required for the generation and recycling of fatty acid to aldehyde.[12] The enzyme Fre (NAD(P)H: flavin oxidoreductase) supplies reduced flavin for the light emitting reaction.[13] The fatty acid reductase complex consists of three components—a fatty acid reductase encoded by *luxC*, an acyltransferase encoded by *luxD*, and an acylprotein synthetase encoded by *luxE*. The proteins LuxE and LuxC are associated in an equal molar ratio in a multienzyme complex[14] that is responsible for the recycling of fatty acid to aldehyde. LuxD catalyses a reaction leading to further production of fatty acid that appears to be decoupled from the main recycling pathway.[15] The reactions catalysed by the products of *luxCDABE* are linked with those of the fatty acid biosynthesis pathway.

The motivation for constructing a mathematical model of the Lux system was the use of the *lux* operon as a reporter system.[16–19] The reporter is constructed by cloning the promoter region of interest into the plasmid, upstream of the *luxCDABE* operon. The promoter controls the expression of the *lux* genes and therefore controls the intensity of the light produced by the LuxAB reaction. The light intensity is therefore a measure of the activity level of the promoter.

The Lux mathematical model could be subjected to different conditions, such as varying protein concentrations, to see whether such changes could be used to optimise the light output from the reporter system. In particular, it could be useful to know which proteins had most control over the steady state concentrations of the system. The model is used to investigate the following: (i) whether such a model could be fitted to the existing experimental data published for the Lux system; (ii) which protein(s) control the light production; (iii) the effects of changing the concentrations of the Lux enzymes; (iv) how best to split the *lux* genes between two plasmids to ensure maximum light production; (v) the sensitivity of the light production rate to changes in parameter values, and in particular to values measured in other bacterial species.

In order to develop the model, values were required for all the kinetic constants involved in the velocity equations. Most of the experimental work on the Lux system has been focused on three bacterial species. The LuxAB reaction has been investigated using *Vibrio fischeri* and *Vibrio harveyi*.[13,20–23] *Photobacterium phosphoreum* has been used for experiments on the LuxC, LuxD and LuxE reactions.[24–28]

Two independent sets of K_m values were available for the LuxAB reaction of *P. luminescens* enzymes.[29,30] K_m and V_{max} values were available from measurements with *V. fischeri* and *V. harveyi* enzymes.[20,23,31–34]

The values for Fre were taken from the work of Inouye and Nakamura,[31] in which H-NMR spectroscopy was used to determine the stereospecificity of the hydride transfer in the Fre reaction. This revealed the mechanism of the reaction and allowed the substrate specificity to be characterised.

The K_m and V_{max} values for the aldehyde substrate of the LuxAB reaction were taken from the work on the dependence of the intensity of the light output on the aldehyde chain length in *Achromobacter fischeri* (*Vibrio fischeri*) by Hastings, Spudich and Malnic.[20] The values were calculated from

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measurements on the pentadecanal and decanal graphs (Fig. 3A and 3B in the paper).

The work of Meighen and Hastings,[23] used kinetic data to determine that there is just one $FMNH_2$-binding site in LuxAB. Both MAV (*Vibrio harveyi*) and Pf (*Vibrio fischeri*) luciferases were studied. The kinetic constants used in the model for $FMNH_2$ were calculated from the two Lineweaver–Burk plots for Pf luciferase (Fig. 8 in the paper), which represented two ranges of $FMNH_2$ concentrations. The time series for the light intensity in the MAV luciferase reaction was used in testing the model.

The kinetic constants for the LuxE reaction were obtained from a study of the fatty acid reductase complex in *Photobacterium phosphoreum* by Rodriguez, Nabi and Meighen.[28] In this study a high pressure liquid chromatographic assay was used to show that the complex consisted of the three polypeptides now known as LuxD, LuxE and LuxC. The values of the kinetic constants were obtained from the Lineweaver-Burk plots for tetradecanoic acid and ATP (Fig. 1 in the paper). The time series in Fig. 3B was also used in testing the model.

LuxC was purified to homogeneity by Rodriguez, Riendeau and Meighen.[27] It was found that in addition to catalysing the reaction of acyl-CoA and NADPH, the enzyme could transfer the acyl group to different thiol reagents in the absence of NADPH. The Lineweaver–Burk plot for the NADPH dependence of tetradecanoyl-CoA was used to obtain the kinetic constants for LuxE.

More detailed information about the graphs used in testing the model is included in the ESI (Lux Data Summary).†

Metabolic control analysis[35,36] has been used successfully to investigate the dependence of the flux through a multi-enzyme system on changes in the concentrations of the component enzymes and substrates.[37] The analysis identifies the extent to which the control of flux is spread in varying proportions between each of the enzymes and substrates in the system. A flux control coefficient is calculated for each enzyme. This is a measure of the rate at which the flux through the system changes as the enzyme activity is changed. It can be expressed in the non-dimensional form

$$C_{E_i}^{J} = \frac{\partial J}{\partial E_i} \cdot \frac{E_i}{J}$$

where J is the pathway flux and $[E_i]$ is the concentration of enzyme i.

The effect on the flux of changing substrate concentrations can be measured for each substrate by the elasticity coefficient:

$$\varepsilon_p^i = \frac{\partial v_i}{\partial p} \cdot \frac{p}{v_i}$$

where v_i is the flux of enzyme i and p is the concentration of the substrate in question.

Methods

Model structure

The chemical equations for the reactions catalysed by the products of the *lux* operon in *Photorhabdus luminescens* are:

$$FMNH_2 + O_2 + RCHO \rightarrow FMN + H_2O + RCOOH + \text{light (490 nm)}$$
(LuxAB — E.C.1.14.14.3)

$$FMN + NADPH + H^+ \rightarrow FMNH_2 + NADP^+$$
(Fre — E.C. 1.5.1.29)

$$RCO\text{-}ACP + H_2O \rightarrow ACP + RCOOH + H^+$$
(LuxD — E.C. 2.3.1.-)

$$RCOOH + ATP + LuxE\text{–}LuxC \rightarrow PPi + AMP + RCO\text{–}LuxE\text{–}LuxC$$
(LuxE — E.C. 6.2.1.19)

$$RCO\text{–}LuxE\text{–}LuxC \rightarrow LuxE\text{–}LuxC\text{–}RCO$$

$$LuxE\text{–}LuxC\text{–}RCO + NADPH \rightarrow RCHO + LuxE\text{–}LuxC + NADP^+ \ (\textit{in vivo})$$
(LuxC — E.C. 1.2.1.50)

$$RCO\text{–}CoA + NADPH + H^+ \rightarrow RCHO + CoA + NADP^+ \ (\textit{in vitro})$$
(LuxC — E.C. 1.2.1.50)

In this paper these are modelled as a system of coupled ordinary differential equations. For this to be done, the set of equations for the steady state velocities of the reactions had to be obtained.

All of the reactions had two or three substrates, so the exact nature of the mechanism of each reaction had to be taken into account in the velocity equations, in particular the order in which substrates bind and products are released. The reactions are modelled as irreversible. The rate equations proposed by Alberty[38] were used. In these equations kinetic parameters are represented by equilibrium constants, rather than rate constants for each step of the reaction as proposed by Dalziel.[39]

The mechanisms employed in each reaction were obtained from the literature.[1,15,22,24,40–44] The relevant steady state equations were found in Segel,[45] Copeland[46] and Cornish-Bowden,[47] where the methods of their derivation included the schematic approach of King and Altman.[48] The method of grouping the rate constants was that of Cleland.[49] For each reaction the version of the velocity equation used was that for the forward reaction in absence of products.

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The velocities are:

$$v = \frac{V_{max}[FMN][NADPH]}{K_{iFMN}K_{mNADPH} + K_{mNADPH}[FMN] + K_{mFMN}[NADPH] + [FMN][NADPH]}$$ **Fre — E.C. 1.5.1.29**

$$v = \frac{V_{max}[FMNH_2][O_2][RCHO]}{\left(\begin{array}{l} K_{iFMNH_2}K_{iO_2}K_{mRCHO} + K_{iO_2}K_{mRCHO}[FMNH_2] + K_{iFMNH_2}K_{mO_2}[RCHO] + \\ K_{mRCHO}[FMNH_2][O_2] + K_{mO_2}[FMNH_2][RCHO] + K_{mFMNH_2}[O_2][RCHO] + [FMNH_2][O_2][RCHO] \end{array}\right)}$$ **LuxAB — E.C. 1.14.14.3**

$$v = \frac{V_{max}[RCO-ACP][H_2O]}{K_{mH_2O}[RCO-ACP] + K_{mRCO-ACP}[H_2O] + [RCO-ACP][H_2O]}$$ **LuxD — E.C. 2.3.1.-**

$$v = \frac{V_{max}[RCOOH][ATP]}{K_{iRCOOH}K_{mATP} + K_{mATP}[RCOOH] + K_{mRCOOH}[ATP] + [RCOOH][ATP]}$$ **LuxE — E.C. 6.2.1.19**

in vivo:

$$v = \frac{V_{max}[LuxE-LuxC-RCO][NADPH]}{K_{mLuxE-LuxC-RCO}[NADPH] + K_{mNADPH}[LuxE-LuxC-RCO] + [LuxE-LuxC-RCO][NADPH]}$$

in vitro: **LuxC — E.C. 1.2.1.50**

$$v = \frac{V_{max}[RCO-CoA][NADPH]}{K_{mRCO-CoA}[NADPH] + K_{mNADPH}[RCO-CoA] + [RCO-CoA][NADPH]}$$

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The velocity equations were combined to produce a set of differential equations to represent the flux through the system of each of the substrates of interest: flavin (FMN), reduced flavin ($FMNH_2$), tetradecanoic acid (represented as RCOOH), tetradecanal (represented as RCHO), and either tetradecanoyl–LuxC–LuxE and tetradecanoyl–LuxE–LuxC (*in vivo*) or tetradecanoyl–CoA (*in vitro*), represented as LuxE–LuxC–RCO, LuxC–LuxE–RCO and RCO–CoA, respectively. The equations are:

$$\frac{d}{dt}[FMNH_2] = v\text{Fre} - v\text{LuxAB}$$

$$[FMNH_2] + [FMN] = F$$

where F is a constant representing the total concentration of flavin and reduced flavin in the system.

Preliminary work revealed that the inclusion of the LuxD reaction makes no difference to the output of the system since the reactions of LuxC and LuxE recycle all of the tetradecanoic acid from the LuxAB reaction. Leaving out LuxD gives the following:

in vivo:

$$\frac{d}{dt}[RCOOH] = -v\text{LuxE} + v\text{LuxAB}$$

$$\frac{d}{dt}[RCO-LuxE-LuxC] = v\text{LuxE} - k_1[RCO-LuxE-LuxC] + k_0[LuxE-LuxC-RCO]$$

$$\frac{d}{dt}[LuxE-LuxC-RCO] = k_1[RCO-LuxE-LuxC] - k_0[LuxE-LuxC-RCO] - v\text{LuxC}$$

where k_1 and k_0 are the rate constants for the forward and back reactions, respectively in the reversible reaction:

$$RCO\text{–}LuxE\text{–}LuxC \rightleftharpoons LuxE\text{–}LuxC\text{–}RCO$$

so

$$[RCOOH] + [RCHO] + [RCO\text{–}LuxE\text{–}LuxC] + [LuxE\text{–}LuxC\text{–}RCO] = R$$

where R is a constant representing the total concentration of fatty acid, aldehyde, acyl–LuxE–LuxC and acyl–LuxC–LuxE in the system.

in vitro:

$$\frac{d}{dt}[RCOOH] = -v\text{LuxE} + v\text{LuxAB}$$

$$\frac{d}{dt}[RCO-CoA] = v\text{LuxE} - v\text{LuxC}$$

so

$$[RCOOH] + [RCO\text{–}CoA] + [RCHO] = R$$

where R is a constant representing the total concentration of fatty acid, acyl-CoA and aldehyde in the system.

Parameter values and testing the model

MAPLE‡ was used to model the sensitivity of the steady state to substrate and enzyme concentrations and kinetic parameter values. Estimates of the limits on the K_m values used in the

‡ http://www.maplesoft.com/

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Table 1 Kinetic parameters used in Lux model

Apparent values			Full model values		
	K_m/μM	V_{max}/μM$_P$ min^{-1} μM$_E^{-1}$		K_m/μM	V_{max}/μM$_P$ min^{-1} μM$_E^{-1}$
LuxAB			**LuxAB**		
Decanal	13.726	0.7441	Decanal	24.5233	1.3297
$FMNH_2$	1.1729	13.06	$FMNH_2$	1.9669	21.904
O_2	0.04	—	O_2	0.0198	
LuxE			**LuxE**		
RCOOH	0.4342	0.6117	RCOOH	0.4342	0.6117
ATP	0.02	0.692	ATP	0.02	0.7522
LuxC			**LuxC**		
NADPH	4.3691	2.2176	NADPH	5.0246	2.5503
RCOCOA	1		RCOCOA	1.0502	
Fixed Concentrations (μM): F = 88 R = 231 NADPH = 560 O_2 = 214 ATP = 1310					

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model were obtained from the enzyme database BRENDA[50] and from the values given in the ESI (Sensitivity to K_m Values).† The sensitivity was expressed as the ratio of the percentage change in substrate concentration to the percentage change in the K_m value.

Generally the velocity of the LuxAB reaction was measured by the intensity of the light generated in the reaction at each time point, so published V_{max} values were expressed in units of quanta per second. For consistency these were converted to units of μM$_P$ min^{-1} μM$_E^{-1}$ (P = product, E = enzyme) using a quantum yield value of 0.2. This was used as an approximate average of the three values of quantum yield for bacterial luciferase found in the literature (0.27,[21] 0.21[51] and 0.164[52]).

For both LuxAB and Fre different values of V_{max} were given for the different substrates in the reactions. These were 'apparent' values of V_{max} since the concentration of one substrate was varied while the other was kept constant. The value appropriate for the substrate being varied in the experiment was used in the model.

The concentrations of the other reactants involved in the system also needed to be estimated. The other reactants estimated and tested were dissolved oxygen, ATP and NADPH. Since the LuxD reaction is omitted from the model, the concentrations of water and RCO–ACP, only associated with the LuxD reaction, were not needed. The environment of the Lux enzymes in typical experiments is that of an *E. coli* cell, so the concentrations of ATP, NADPH and FMN found in an *E. coli* cell were used.[37] The values were 1310 μM for ATP, 560 μM for NADPH, and 88 μM for FMN. The value of the concentration of dissolved oxygen in the cell was not available, so its concentration in water at 37 °C at an atmospheric pressure of 101.3 kPa was calculated,§ giving a value of 214 μM. The values for ATP, NADPH and oxygen were taken as fixed within each simulation, and the sensitivity of the steady state light intensity to the values of these concentrations was analysed by varying each of them over five orders of magnitude. The results are included in the ESI (Sensitivity to Substrate Concentrations).†

The accuracy of the model was tested by comparing its output against the results from published experiments on the Lux system. One difficulty with this was that the LuxE and LuxC reactions *in vivo* are thought to involve the transfer of the tetradecanoyl group from LuxE to LuxC, although the process is not well understood.[44] This is shown in the statement of the chemical equations for LuxE and LuxC. The *in vitro* experiments in the literature, however, use the fact that LuxC can also reduce other activated fatty acids such as acyl-CoA.[26] In many papers tetradecanoyl-CoA is used in assays for LuxC,[27] so the steady state velocity equation for LuxC would have to be in the *in vitro* form if the model was to be tested against these results.

The system of equations was solved in two ways, each employing the kinetic parameters and fixed concentrations listed in Table 1. Firstly, each of the *in vivo* differential equations were set to zero and the system was solved to obtain the steady state concentrations of the six products and the rate at which light was produced in this state. Secondly, the *in vitro* equations in velocity form were used to obtain a time-dependent solution for the concentration of each of the five products being investigated, and the intensity of the light being produced.

The model was tested by being used to reproduce time series data from papers using the published parameter values, and to reproduce stationary data from concentration curves.

To improve the accuracy of the steady state concentration values and the fit to the time series data, estimates for the K_m and V_{max} values for each set of stationary data were obtained by using the nonlinear least squares data fitting routine in MATLAB¶ on a series of data points measured from a suitable graph in the literature. Each data set and corresponding graph showed the output from the simulation of an experiment involving a single enzyme: LuxAB, LuxE or LuxC. These estimates were used as parameters in a Michaelis–Menten equation, which was plotted on the same graph as the relevant set of data points. The time series data were also plotted and compared with the curve obtained using the relevant K_m and V_{max} values.

The K_m and V_{max} values used in the model fitting are apparent values, and needed to be converted to values appropriate for the model using suitable sets of simultaneous equations. The flux control coefficient was calculated for each enzyme held at the concentration used in the relevant experiment. The variation in flux control coefficient values with enzyme concentration was examined. The kinetic parameters used in the model are shown in Table 1 above.

§ http://antoine.frostburg.edu/chem/senese/101/solutions/faq/predicting-DO.shtml

¶ http://www.mathworks.com/

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Results and Discussion

There is good agreement between the model and experimental data

Fig. 2 shows the results of fitting the model to the published experimental data.

For LuxAB, the model is in good agreement with the graphs for both pentadecanal and decanal, with the fit for decanal being slightly better than that for pentadecanal. The main graph for $FMNH_2$ spans a lower range of concentrations than the small graph, and shows slightly better fit.

The time series for rate of AMP formation with LuxE follows the Michaelis–Menten curve only approximately, with similar initial velocity and asymptotic value, but smoother shape. The reason for the discrepancy may be that in the experiment LuxC was also present.

There is, however, no correlation of the model with the LuxAB time series. On a log scale the rate of light production decays at a rate of 0.1 s^{-1}. The model has a log decay rate of 3.04×10^{-5} s^{-1}.

The most likely explanation for the lack of agreement of the model and the LuxAB time series data is that the fast decay in the experimental time series results from the dark pathway. To test this hypothesis, the equation for the total decay rate was applied to data from studies on *Photobacterium phosphoreum*.[25]

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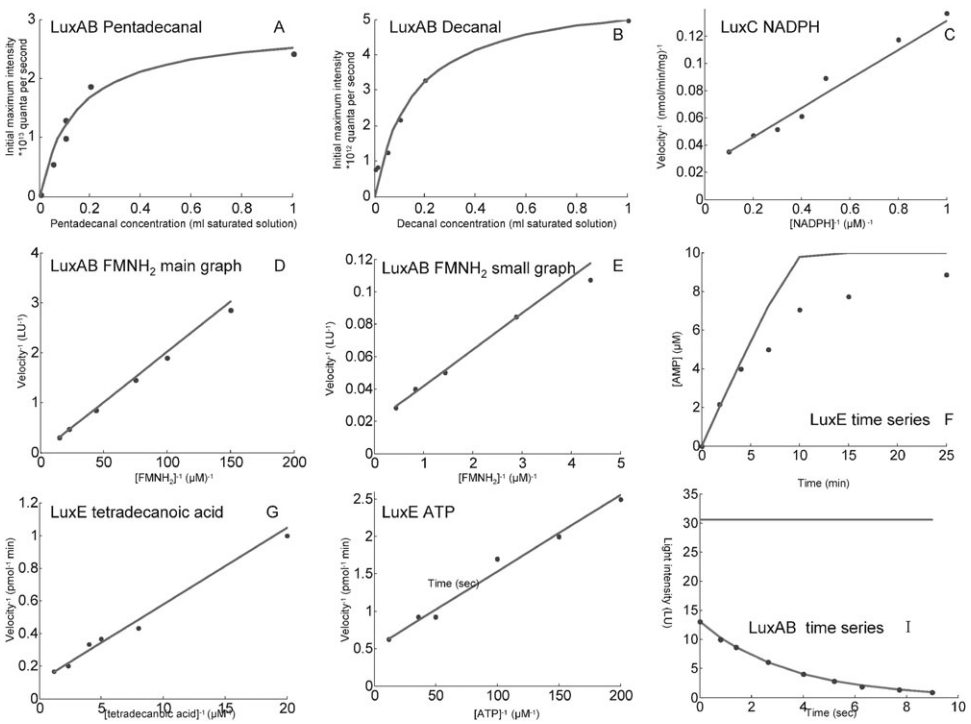

Fig. 2 Comparisons between model predictions and experimental observations. Each graph displays the velocity of the reaction and the substrate concentrations in the units used in the relevant paper. A,B. Effect of aldehyde concentration on the velocity of the luminescence reaction. The velocity is measured by the initial intensity of the bioluminescence. The concentrations are expressed in millilitres of a saturated solution of aldehyde in a final volume of 2.0 ml. Both graphs show a very good fit of the model to the data. (Data obtained from Fig. 3, Hastings *et al.*, 1963.[20]) C. Lineweaver–Burk plot for the NADPH dependence of LuxC activity. The velocity of the LuxC reaction is measured from the stimulation of luciferase activity by the aldehyde product of the reaction. It is expressed as nanomoles of aldehyde produced per minute per milligram of Lux C. Again the model fits well to the data. (Data from Fig. 2S, Rodriguez *et al.*, 1983.[27]) D,E. Lineweaver–Burk plots for the dependence of the initial light intensity of the LuxAB reaction on the concentration of $FMNH_2$. The light intensity is measured in light units (LU), where 1LU is equivalent to 2.2×10^{10} quanta per second. There is a close fit to the data over two separate ranges of concentration. (Data from Fig. 8, Meighen and Hastings, 1971.[23]) F. Time series of an assay for ATP hydrolysis using a mixture of LuxE and LuxC. The velocity is measured by the rate of formation of AMP, the product of the LuxE reaction, using a high performance liquid chromatography assay. It can be seen that the model has the same initial velocity and asymptotic value as the data. However, the data is more smoothly saturating than the model, which is based on Michaelis–Menten kinetics. This indicates that there is probably a more complex mechanism involved in the experiment. It is possible that the presence of LuxC has some influence on this, but the LuxC reaction which forms part of the luminescent recycling system requires the presence of NADPH. The data set used here was for a reaction mixture which did not contain NADPH. (Data from Fig. 3B, Rodriguez *et al.* 1985.[43]) G,H. Lineweaver–Burk plots for the tetradecanoic acid and ATP dependence of LuxE. The velocity is measured by including LuxC in the reaction mixture and coupling this to luciferase. The velocity is given as the rate in picomoles per minute at which aldehyde is produced from the luminescent reaction. As with the other sets of stationary data, the model shows a very good fit. (Data obtained from Fig. 1, Rodriguez *et al.* 1985.[43]) I. Time series for decay of the LuxAB reaction in *Vibrio harveyi*. The velocity is measured by the intensity of the luminescent reaction in light units as defined for Fig. D and E. The intensity initially rises sharply, then decays at a constant rate of 0.1 s^{-1} on a log scale. (Data from Fig. 2, Meighen and Hastings 1971.[23]) The rate for the model without the dark reaction is 3.04×10^{-5} s^{-1}; this model does not fit the data. The green line is an exponential decay with a constant rate of 0.1 s^{-1} on a log scale, that would reflect the inclusion of a dark reaction (in which decay occurs without emission of light) with a k_D of 0.0931 s^{-1}. Therefore the inclusion of the dark reaction can explain these experimental time series data.

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For these data,

$K_A = 2.8 \times 10^{-4}$ M, $k_L = 0.15$ s^{-1} and $k_D = 0.25$ s^{-1}.

The total decay rate is therefore

$$k_T = (0.15 \times A + 0.25 \times 2.8 \times 10^{-4})/(2.8 \times 10^{-4} + A)$$

In the LuxAB time series used to test the model, the aldehyde concentration was 0.384×10^{-4} M. Using this in the k_T equation gives a total decay rate of 0.2379 s^{-1}. The actual decay rate was 0.1 s^{-1}. The discrepancy may be explained by the fact that bacterial luciferases can be placed into two distinct categories, 'fast' and 'slow'. The time series was for *V. harveyi*, which is classed as 'slow', whereas P. phosphoreum is classed as 'fast'. 'Fast' luciferases have high values of K_A and a rapid decay through the dark pathway. For example, with the same values of K_A and k_L, the observed decay rate of 0.1 s^{-1} could be explained with $k_D = 0.0931$ s^{-1}.

Control of the system lies mainly with LuxE and LuxC

Fig. 3A shows the light output of the system as a function of the rate constant of the reaction in which an acyl group is transferred from LuxE to LuxC. It can be seen that the light output rises from close to zero to 1.8×10^{-3} μM min^{-1} over a range of k values of three orders of magnitude. For values of k above 0.1, the light output is constant.

The total concentration of the LuxEC dimer (denoted 'LuxECtotal') is divided between three forms in the system: free enzyme (LuxECfree), with an acyl group bound to LuxE,

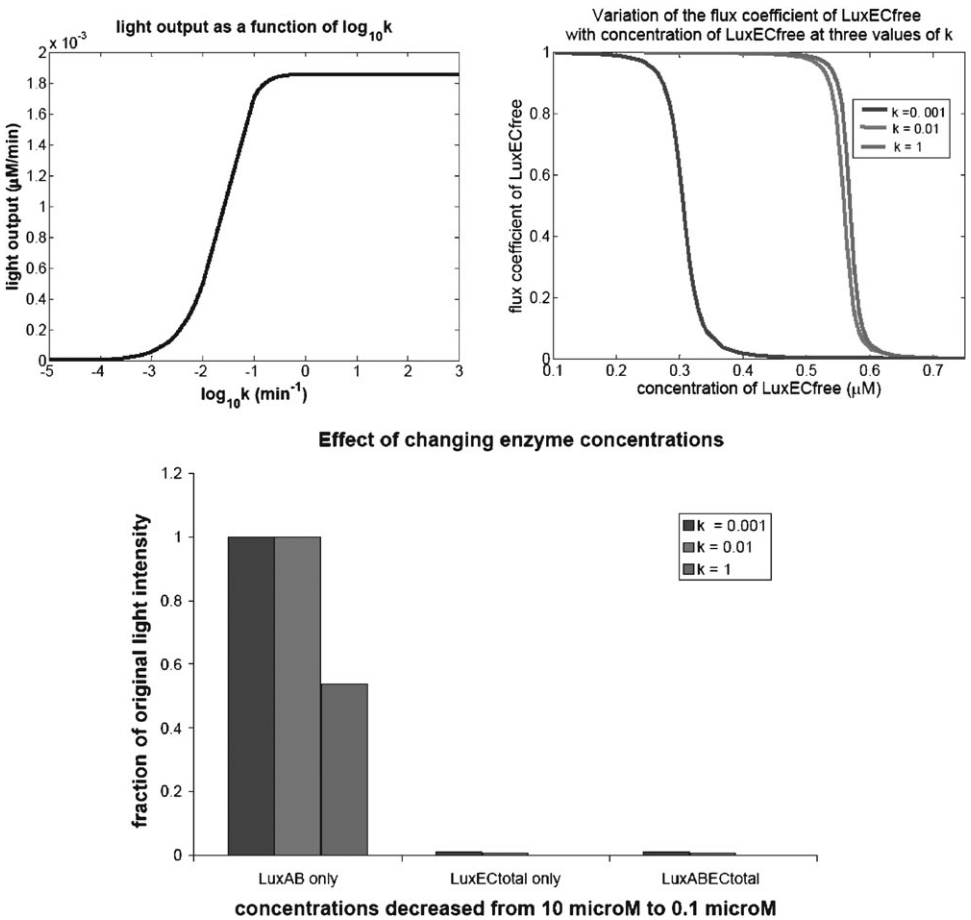

Fig. 3 Change of control with enzyme concentration in the recycling pathway. A. The light output of the system as a function of the rate constant of the reaction in which an acyl group is transferred from LuxE to LuxC. It can be seen that the light output rises from close to zero to 1.8×10^{-3} μM min^{-1} over a range of k values of three orders of magnitude. The total concentration of the LuxEC dimer (denoted 'LuxECtotal') is divided between three forms in the system: free enzyme (LuxECfree), with an acyl group bound to LuxE, and with an acyl group bound to LuxC. B. The variation of the flux coefficient of LuxECfree with the concentration of LuxECfree. It can be seen that the control of LuxECfree over the system extends to higher concentrations as the value of k increases. The concentration of LuxECtotal used in the system is 0.0588 μM. This corresponds to a LuxECfree concentration of 8.5×10^{-5} μM. Even with k as low as 0.001 min^{-1}, the control coefficient at this concentration is almost 1. C. A summary of the effects on the steady state light intensity of changing the concentrations of the Lux enzymes, in a way that might correspond to placing different groups of genes under the control of an inducible plasmid. Each set of bars corresponds to a different enzyme or combination of enzymes. The colour of the bar indicates the value of k used in that simulation. The height of the bar indicates the fraction to which the light intensity is reduced when the concentration of the enzyme(s) indicated decreases from 10 μM to 0.1 μM. The concentrations of the other enzymes remain at 10 μM. Part C shows that if the concentration of LuxAB only is reduced, the light intensity does not change if k is 0.001 or 0.01, but reduces to almost half if k is 1. If the total concentration of LuxEC is decreased, with or without a reduction in LuxAB, the light intensity reduces to less than 1% at all values of k, the reduction being greatest for $k = 1$.

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and with an acyl group bound to LuxC. Fig. 3B shows the variation of the flux coefficient of LuxECfree with the concentration of LuxECfree. It can be seen that the control of LuxECfree over the system extends to higher concentrations as the value of k increases.

The concentration of LuxECtotal used in the system is 0.0588 μM. This corresponds to a LuxECfree concentration of 8.5×10^{-5} μM. Even with k as low as 0.001 min^{-1}, the control coefficient at this concentration is almost 1. This is further evidence that LuxEC has almost total control over the light output of the system.

Fig. 3C shows a summary of the effects on the steady state light intensity of changing the concentrations of the Lux enzymes, in a way that might correspond to placing different groups of genes under the control of an inducible plasmid. Each set of bars corresponds to a different enzyme or combination of enzymes. The colour of the bar indicates the value of k used in that simulation. The height of the bar indicates the fraction to which the light intensity is reduced when the concentration of the enzyme(s) indicated decreases from 10 μM to 0.1 μM. The concentrations of the other enzymes remain at 10 μM.

Fig. 3C shows that if the concentration of LuxAB only is reduced, the light intensity does not change if k is 0.001 or 0.01, but reduces to almost half if k is 1. If the total concentration of LuxEC is decreased, with or without a reduction in LuxAB, the light intensity reduces to less than 1% at all values of k, the reduction being greatest for $k = 1$. This is further evidence that control of light production lies within the recycling path rather than in the luminescent reaction, and suggests that LuxEC should be inducible, but LuxAB and Fre should be constitutively expressed (Changing the concentration of Fre has already been shown to have no effect on light intensity).

The steady state light production rate shows a low sensitivity to changes in parameter values

The data concerning the sensitivity of the steady state of the model to its K_m values is included in the ESI.† It was found that most of the K_m values for the four species covered by the available literature differ considerably from those used in the model, but the resulting light production rate only changed by at most 0.03%. Where a range of observed K_m values were not available from the literature, we tested sensitivity using a range of K_m over several orders of magnitude. The largest percentage change in light production rate using these additional values was −4%. This was for an increase in the K_m value used in the model by a factor of 25.

The analysis of the sensitivity of the steady state light intensity to the values of the concentrations of ATP, NADPH and oxygen showed that for seven combinations of concentrations varying over five orders of magnitude the light intensity was changed at most by 1.5%, demonstrating that the model is not particularly sensitive to the values of these parameters.

Concluding remarks

We have constructed a differential equation model for the Lux luminescence system. We have tested the model on published experimental data from several sources, and found good agreement. We have used metabolic control analysis to show that the control of the system lies mainly with the enzymes LuxE and LuxC. This conclusion is also supported by the results of using the model to show how changes in enzyme concentrations affect the steady state light intensity.

A prediction from this study is that a reporter system constructed from the *luxABCDE* operon of *P. luminescens*, expressing LuxAB constitutively, and with the promoter under study in front of *LuxCDE*, should act as an efficient reporter; this might have the advantage of speeding up the response time. The reverse configuration should not work. However, the utility of these ideas would need to be investigated through experiment.

The model we construct considers the dynamics of the luminescence system itself and does not incorporate the kinetics of protein synthesis and degradation. There are two reasons for this. First, we are interested in constructing a model that is explanatory of *in vitro* data, where these processes are not relevant. Second, *in vivo* protein synthesis and turnover will depend on the promoter under study, the host organism and experimental conditions. The advantage of our approach is that we develop and analyze a generic chemical model that can be embedded into larger models for specific applications that also incorporate the protein dynamics.

Thus this study lays the foundation for future work that would allow powerful analysis of data from Lux reporter experiments. In particular, such work could include the reverse engineering of promoter activity from luminescent readout. This would require not only a sophisticated model in the form of the one developed here, but also additional parameters that would need to be experimentally defined, including the rates of synthesis of Lux proteins and their molecular stability.

Acknowledgements

We thank Neil Burton, Dafyd Jenkins, Jan Kreft, Peter Lund, Helen Parsons, and Chris Wharton for invaluable discussion and technical help. PAW is funded by a BBSRC Targeted Priority Research Studentship (BBS/S/E/2006/13186).

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