



## 23 Abstract

24 The bacterial Lux system is used as a gene expression reporter. It is fast, sen-  
25 sitive and non-destructive, enabling high frequency measurements. Originally  
26 developed for bacterial cells, it has been adapted for eukaryotic cells, and can  
27 be used for whole cell biosensors, or in real time with live animals without the  
28 need for slaughter. However, correct interpretation of bioluminescent data is  
29 limited: the bioluminescence is different from gene expression because of non-  
30 linear molecular and enzyme dynamics of the Lux system. We have developed  
31 a modelling approach that, for the first time, allows users of Lux assays to infer  
32 gene transcription levels from the light output. We show examples where a de-  
33 crease in bioluminescence would be better interpreted as a switching off of the  
34 promoter, or where an increase in bioluminescence would be better interpreted  
35 as a longer period of gene expression. This approach could benefit all users of  
36 Lux technology.

## 37 Introduction

38 The lux operon contains genes for the bacterial bioluminescent reaction[1, 2]:  
39 *luxA* and *luxB* encode the  $\alpha$  and  $\beta$  subunits of the heterodimeric bacterial lu-  
40 ciferase; *luxC* encodes a 54kDa fatty acid reductase; *luxD* encodes a 33kDa acyl  
41 transferase; and *luxE* encodes a 42kDa acylprotein synthetase. These genes,  
42 including their order (*luxCDABE*), are conserved in all lux systems of biolumi-  
43 nescent bacteria. An additional gene (*luxF*), with homology to *luxA* and *luxB*,  
44 is located between *luxB* and *luxE* in some species. The light emitting reaction,  
45 catalysed by the LuxAB complex, involves the oxidation of FMNH<sub>2</sub> and the  
46 conversion of a long chain fatty aldehyde (tetradecanal *in vivo*) to its cognate  
47 acid, with the emission of blue-green light. LuxC, D and E together form the  
48 fatty acid reductase complex, involved in a series of reactions that recycle the  
49 fatty acid back to aldehyde. In *E. coli* and other species, Fre has been shown  
50 to be the enzyme responsible for flavin reduction back to FMNH<sub>2</sub>.

51 Gene expression can be measured by cloning a promoter of interest upstream  
52 of the lux operon, and interpreting the bioluminescence from bacteria containing  
53 such constructs as a measure of transcription[3, 4]. This provides a reporter  
54 that can measure gene expression at high frequency and with less background  
55 noise than other reporters, such as GFP[5, 4], and has found great value in  
56 both bacteria [6] and eukaryotes[7], with important recent applications in whole

57 cell biosensors[8], live animal infection models[9, 10] and live tumour infection  
58 models[11].

59 However, this light is an integrated signal of transcription, mRNA half-  
60 life, translation and protein turn-over, the bioluminescence reaction kinetics  
61 and substrate availability and cycling. As a consequence, absolute transcription  
62 activity cannot be directly inferred from the data generated. This is a limitation  
63 in the current use of Lux technologies. For example, some studies using Lux  
64 reporters have observed fluctuations in light output[12, 13, 14, 15, 16], and it  
65 is not clear whether these truly reflect promoter activity, or are artefacts of the  
66 reporter system.

67 In this paper we show that detailed mathematical models for bioluminescence  
68 can be used to relate bioluminescence to promoter activity. We developed a  
69 new model that addresses limitations of previous work[17], whose structure and  
70 parameters are informed by new data and new mechanisms. We generated new  
71 enzymatic data both for the Fre reaction and for the LuxAB reaction, using  
72 *Photorhabdus luminescens* LuxAB that we have purified. An important part  
73 of our approach is to fit the models directly to the time series experimental  
74 data, which can be thought of as a (partial) factorial experiment with varying  
75 concentrations of both FMN and NADPH. This allows for the simultaneous  
76 inference of all parameters in complex kinetic models, which is an advantage  
77 over traditional chemical kinetics techniques that only use the maximal velocity.

## 78 Results and Discussion

79 The luciferase reaction uses FMNH<sub>2</sub> as an energy source. This is an ener-  
80 getically transient species with a short half-life. *In vivo* it is supplied to the  
81 luciferase complex for immediate consumption by the redox activity of the Fre  
82 enzyme, which converts NADPH and FMN to FMNH<sub>2</sub> and NADP<sup>+</sup>. We mea-  
83 sured the kinetics of the Fre reaction (Figure 1a) by recording the consumption  
84 of NADPH (as determined spectrophotometrically) at different starting concen-  
85 trations of the FMN acceptor component (100μM, 200μM and 400μM); in all  
86 cases, the initial NADPH concentration was 200μM. The initial velocity of the  
87 reaction increased with increased FMN, leading to different kinetics in the three  
88 curves, with the steady state being reached more rapidly with increased FMN  
89 concentration.

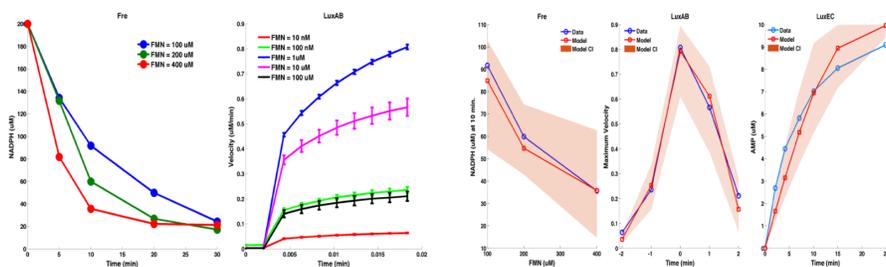


Figure 1: (a) New experimental data for Fre and LuxAB reactions. NADPH time course for three different concentration of FMN. The concentration values in the above data are obtained from the absorption measurements from the spectrophotometer. The conversion is carried out by relating the concentration ( $C$ ) to the measured values ( $A$ ) with the formula  $A = K * C$ , where  $K$  is the proportionality constant and is estimated using initial measurement ( $A_0$ ) and starting concentration ( $C_0 = 200\mu M$ ). The velocity of the reaction increases as FMN concentration is increased. Normalised (using max. velocity in the assay) LuxAB reactions velocity time series for different FMN concentrations. The velocity of the reaction increases from the lowest concentrations of FMN, is greatest for FMN concentrations of  $1\mu M$ , and decreases for higher concentrations of FMN. This is best explained by product inhibition of the LuxAB reaction through competition between FMN and the substrate  $FMNH_2$ . (b) Model fits for Fre, LuxAB, and LuxEC reactions. The model fits to the data are good, showing that kinetic parameters for the reaction rates can be inferred. Summarized data are displayed: for Fre - NADPH concentrations at  $t = 10$  min; for LuxAB, the maximal velocity for each FMN concentration; for LuxEC - only the AMP time-course data are shown. The full data fits for all three reactions are shown in supplementary figures. Total flavin =  $88\mu M$ ,  $O_2 = 550\mu M$ ,  $NADPH = 560\mu M$ , and  $ATP = 1310$ .

90 For the measurement of luciferase (LuxAB) kinetic reactions, we combined  
 91 components of the coupled Fre-LuxAB reaction. We measured light output  
 92 arising from different initial concentrations of FMN: 10nM, 100nM,  $1\mu M$ ,  $10\mu M$   
 93 and  $100\mu M$  (Figure 1a). For all five conditions, there is an initial delay before  
 94 light is produced, due to a two-step injection method. Following the lag, light  
 95 is produced, initially rapidly, and then tailing off. A striking feature of these data  
 96 is that maximum light production increases as FMN concentration is increased  
 97 from 10nM to  $1\mu M$ , but then decreases again as FMN concentration is increased  
 98 further. The most likely explanation for this decrease is inhibition of the LuxAB  
 99 reaction by its product, FMN, which would be competing with  $FMNH_2$  for  
 100 binding to the LuxAB complex. It cannot be substrate inhibition of Fre by  
 101 FMN as the kinetics for this reaction increase up to  $400\mu M$  (Figure 1a). Product

102 inhibition has not been previously reported and represents a newly discovered  
 103 element of the Lux bioluminescent pathway. Structural studies have shown that  
 104 both FMN and FMNH<sub>2</sub> can form a complex with LuxAB[18], with only two  
 105 residues that could act to discriminate between them (see PDB 3fgc), supporting  
 106 the proposed product inhibition.

107 The new mathematical model includes three chemical reactions: the Fre  
 108 reaction for flavin recycling; the LuxAB reaction for light production; and the  
 109 LuxEC reaction for aldehyde recycling. It also contains a further equation to  
 110 describe the turnover dynamics of the Lux proteins:

$$\begin{aligned} \frac{d[\text{FMNH}_2]}{dt} &= v_{Fre}Fre - v_{LuxAB}LuxAB \\ \frac{d[\text{FMN}]}{dt} &= -v_{Fre}Fre + v_{LuxAB}LuxAB \\ \frac{d[\text{RCOOH}]}{dt} &= v_{LuxEC}LuxEC + v_{LuxAB}LuxAB - \tau[\text{RCOOH}] \\ \frac{d[\text{RCHO}]}{dt} &= \rho_0 - v_{LuxAB}LuxAB + v_{LuxEC}LuxEC - \tau[\text{RCHO}] \\ \frac{dP}{dt} &= T(t) - \gamma_L P \\ [\text{FMNH}_2] + [\text{FMN}] &= F \\ [\text{RCOOH}] + [\text{RCHO}] &= R_0 \end{aligned}$$

111  
 112 It is assumed that the action of LuxD counterbalances the loss of RCOOH  
 113 and RCHO; thus  $R_0$  is constant and  $\rho_0 = \tau R_0$ . Control of the bioluminescent  
 114 reactions is assumed to lie with the most rapidly turned-over protein. This is  
 115 modelled by setting  $P(t)$  to represent all of the Lux proteins and using the same  
 116 rate of synthesis and turn-over for all proteins. The protein production  $T(t)$   
 117 encompasses transcription, translation and mRNA degradation and is supplied  
 118 as a model input; it is assumed that the *lux* mRNA is at quasi steady state.  
 119 The value of the protein turn-over rate  $\gamma_L$  has been inferred from Lux data as  
 120  $0.378\text{h}^{-1}$  (Figure 2). Full details for the velocity equations for the terms  $v_{Fre}$ ,  
 121  $v_{LuxAB}$  and  $v_{LuxEC}$  are provided in the Supplementary Materials. New detailed  
 122 mechanisms have been defined using King and Altman's schematic method [19],  
 123 with a modification to the  $v_{LuxAB}$  velocity term to include the impact of FMNH<sub>2</sub>  
 124 product inhibition.

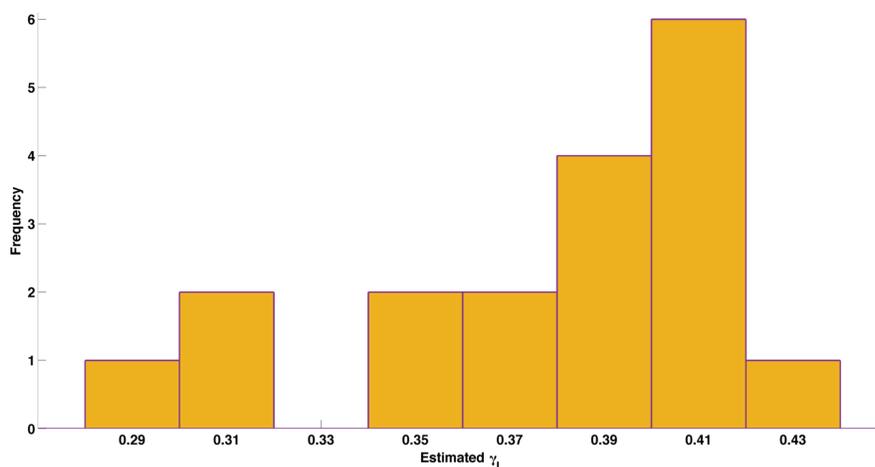


Figure 2: Histogram of inferred Lux protein turnover rates, showing low variability about a mean value of  $0.378\text{h}^{-1}$ .

125 We have carried out parameter inference for all three reactions: Fre, LuxAB  
126 and LuxEC, using a Markov Chain Monte Carlo (MCMC) approach, as in previ-  
127 ous work[20, 21]. The results are posterior distributions for each of the param-  
128 eters in the model, which describe not only the best fit values for the param-  
129 eters, but also the degree of uncertainty of the parameter estimates. Because the Fre  
130 reaction is also used in the LuxAB experiment, inference for these parameters  
131 was carried out in two steps: first we fitted the experimental data from the  
132 Fre reaction to produce posterior distribution for these parameters; then we  
133 used these posterior distributions as prior distributions for the same param-  
134 eters in the LuxAB reactions. For the LuxEC reactions, we used published data  
135 on steady-state measurements of NADPH, ATP and RCOOH, as well as time-  
136 course measurements of AMP formation[22, 23, 1], as shown in Figure 2 (B,F,G,  
137 H) of Welham *et al.*[17]. There is good concordance between the model and  
138 the data (Figure 1b), with the time course in particular showing substantial im-  
139 provement ( $R^2 = 0.762$ ) over the model fit previously reported[17] ( $R^2 = 0.396$ ),  
140 consistent with improved model mechanism and parameter inference. Detailed  
141 fitted curves and MCMC diagnostics are shown in Supplementary Materials,  
142 and the inferred parameters are given in Supplementary Table 1.

143 A key finding of this analysis, of particular significance for inferring levels  
144 of gene expression with lux reporters, is that there is a nonlinear relationship  
145 between promoter activity and light output (Figure 3). Bioluminescent outputs

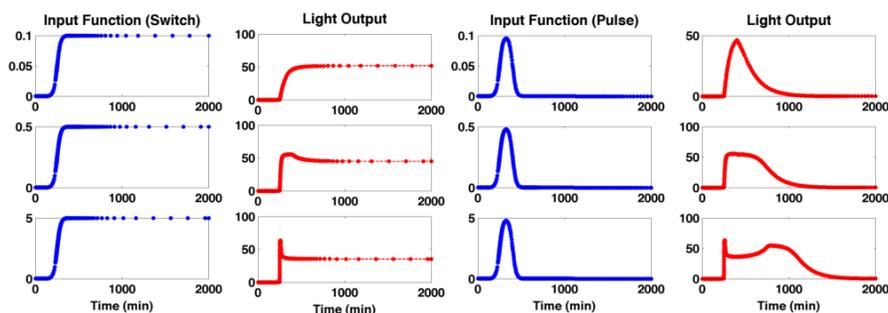


Figure 3: Relationship between promoter activity and light output Nonlinear relationship between promoter activity and light output for a synthetic pulse or switch of gene expression at different levels. The bioluminescence displays different qualitative and quantitative behaviours from the underlying gene expression. With the switch data, the bioluminescence has slower onset compared with gene expression, and, for high levels of gene expression, shows a transient pulse not present in the gene expression. With the pulse data, the bioluminescence shows much longer persistence than the underlying gene expression. These data show that bioluminescence alone could be a misleading measure of gene expression.

146 display qualitative and quantitative behaviours different from the underlying  
147 gene expression, so direct interpretation of bioluminescent data can be mislead-  
148 ing. Simulations of the model for a switching on of gene expression show that for  
149 low levels of expression, bioluminescence appears more gradually with a slight  
150 delay, while for higher levels of gene expression there is a transient peak of bi-  
151 oluminescence followed by lower steady state. Simulations for a transient pulse  
152 of gene expression also produce bioluminescent outputs that are different from  
153 the underlying gene expression. Bioluminescence decreases more slowly than  
154 gene expression, and, for higher levels of gene expression, bioluminescence can  
155 remain high long after gene expression has ceased. More detailed plots of all  
156 species in the reactions can be found in Supplementary Materials.

157 The model can be used to reverse engineer gene expression from biolumi-  
158 nescence, also using an MCMC approach. We have tested this approach on  
159 three datasets: the synthetic pulse data set, where the underlying gene ex-  
160 pression to be inferred is supplied; data from the uhpT promoter in *S. aureus*,  
161 with relatively simple light profiles; and data from an acid stress experiment in  
162 *E. coli*, with more complicated bioluminescent output, for the promoter of the  
163 *safA-ydeO* operon in four strains following exposure to acid: wild-type, a *ydeO*

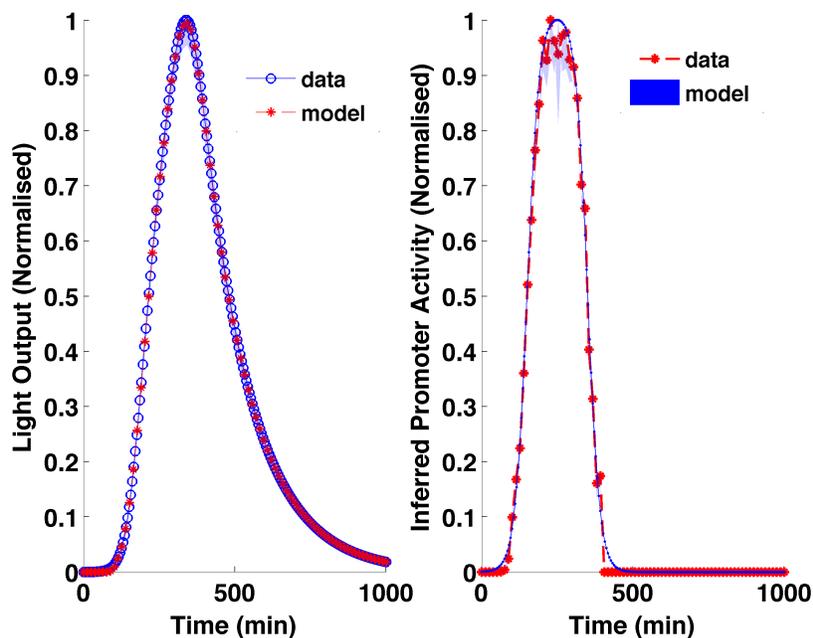


Figure 4: Reverse engineered promoter activity from light output from a simulated transient pulse experiment showing effective and accurate recovery of the known gene expression profile.

164 knockout, a *phoP* knockout and a *ydeOphoP* double knock out; both YdeO and  
165 PhoP repress this promoter[15].

166 The reverse engineering of the synthetic pulse data show accurate repro-  
167 duction of the supplied input gene expression profile, demonstrating that our  
168 method works correctly (Figure 4). For the *uhpT* data (Figure 5a), the inferred  
169 promoter activity suggests both earlier gene expression and more rapid switch-  
170 ing off of expression than would be apparent from the light output. The most  
171 profound difference in interpretation of results based solely on levels of emitted  
172 light arise from the acid stress data (Figure 5b). While the inferred gene expres-  
173 sion is less smooth than the bioluminescence, it highlights three behaviours not  
174 apparent in the bioluminescence itself. First, there is a clear, transient pulse  
175 of gene expression following acid stress, lasting only 20 minutes in the WT, 30  
176 minutes in the single knockout strains, and 40 minutes in the double knock-out.  
177 In the WT and *ydeO* mutant, gene expression is completely switched off after  
178 this pulse, which cannot be seen from the bioluminescence, while in the *phoP*

179 and double mutants, it is not completely switched off. Second, the increase in  
180 gene expression in the mutants relative to the WT is much lower than indicated  
181 by the bioluminescence, with the increased bioluminescence reflecting increased  
182 duration of gene expression as much as increased level. Third, the inferred gene  
183 expression appears to show pulses. These reflect the experimental protocol, in  
184 which plates were moved between the luminometer and the spectrophotometer  
185 every 15 minutes, disturbing the cells. The pulses suggest that the protocol  
186 had a more profound impact on cell activity than would be apparent from the  
187 light. The peaks of these pulses are more likely to represent gene expression  
188 level, indicative of a long term steady state gene expression, also not apparent  
189 from the bioluminescence. While the transient gene expression of the *safA-ydeO*  
190 promoter is to be expected in the WT and two single mutant strains, the reason  
191 for its transience in the double mutant, i.e. in the absence of the two known  
192 down-regulators of the promoter, is not known. The promoter is activated by  
193 the EvgA response regulator following a drop in pH, and the kinetics of turnover  
194 and dephosphorylation of this activator are unknown. They may explain the  
195 transient activation of the promoter, or there may be other feedback systems  
196 operating. Other known acid responsive regulators such as GadE, GadX and  
197 GadW are not responsible, as their deletion has no affect on promoter induction  
198 kinetics[15].

199 In conclusion, we have developed a new mathematical model to relate gene  
200 expression to light output in Lux promoter assays, that includes newly discov-  
201 ered experimental evidence for product inhibition of the LuxAB reaction by  
202 FMNH<sub>2</sub>. The model shows a nonlinear relationship between gene expression  
203 and light output. We have used the model to provide a method to infer gene  
204 expression from light output that can be generally applied to experiments using  
205 Lux reporter assays. We anticipate that this approach could have valuable ap-  
206 plications in inferring gene expression levels in a wide range of biological systems  
207 where lux reporters can be employed, including both *in vitro* experiments, and  
208 to track gene expression in animal models of bacterial infection. Program code  
209 to undertake reverse engineering of promoter activity are provided as Matlab  
210 and R functions in supplementary files.

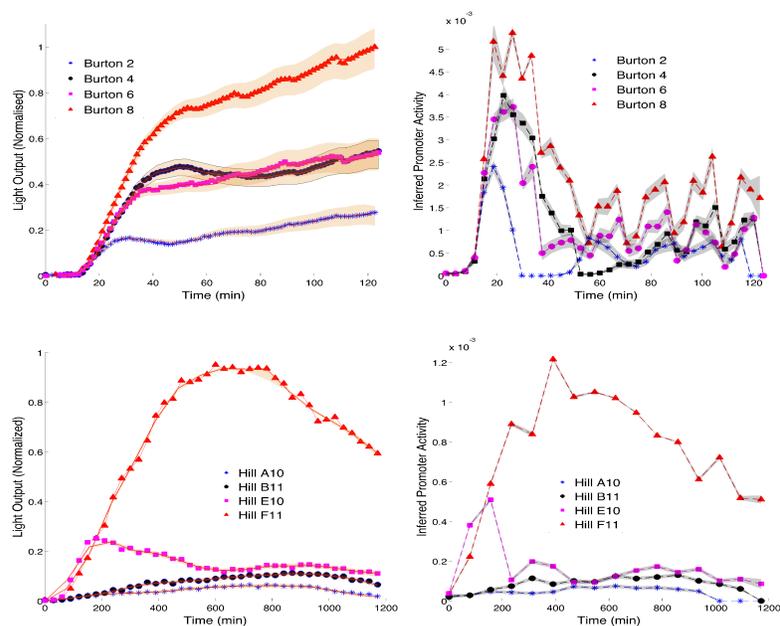


Figure 5: (a) Reverse engineered gene expression from data from the *uhpT* promoter in *S. aureus* showing that the peak of gene expression occurs earlier than the light output, and that the duration of gene expression is shorter than would appear from the light output. (b) Reverse engineered gene expression from the *safA-ydeO* promoter in *E. coli*. The pattern of gene expression is very different from the light output pattern. In particular, the increased bioluminescence is partly explained by greater duration of gene expression not just level of gene expression; gene expression in the WT and *yodel* mutant appears to be switched off after induction, which is not apparent in the bioluminescence; and the inferred expression shows pulses which are an artefact of the experimental arrangement, in which plates were moved every 15 minutes between a spectrophotometer and a luminometer, demonstrating that the agitation has an impact upon the cells.

## 211 **Methods**

### 212 **Chemicals, media, cloning, bacterial strains**

213 All chemicals were purchased from Sigma-Aldrich unless indicated otherwise,  
214 and were of ultrapure quality. Routine cloning steps were carried out using stan-  
215 dard molecular biological protocols. Primary clones were selected in LB with  
216 Mach I Electrocompetent or chemically *E. coli* obtained from Thermo-Fisher  
217 Scientific. Protein expression was carried out using the *E. coli* strain BL21  
218 (DE3) which had been previously transformed with the arabinose inducible plas-  
219 mid pGRO7, encoding the GroES-GroEL chaperone complex (Takara Inc.). Au-  
220 toinduction medium was used for the protein purification steps (Overnight Ex-  
221 press Autoinduction system 1 — Novagen). See below for further details of the  
222 luciferase purification strategy. Antibiotic selection was carried out at the fol-  
223 lowing concentrations: Chloramphenicol -  $20\mu\text{gml}^{-1}$ , Carbenicillin -  $100\mu\text{gml}^{-1}$ ,  
224 Kanamycin -  $50\mu\text{gml}^{-1}$ .

### 225 **Measurement of Fre activity**

226 The commercially available Fre/NAD(P)H:FMN-Oxidoreductase of *P. lumi-*  
227 *nescens* was obtained from Roche Diagnostics. The activity of the enzyme was  
228 determined using a continuous spectrophotometric rate determination method  
229 as follows: NADPH ( $200\mu\text{M}$ ), FMN ( $100\text{-}400\mu\text{M}$ ) and Fre ( $5\mu\text{l}$  of a  $0.2$  unit  
230  $\text{ml}^{-1}$  preparation per ml final reaction mix) was prepared in  $50\text{mM}$  potassium  
231 phosphate buffer, pH 7.0. Replicate  $1\text{ml}$  samples were prepared in sterile cu-  
232 vettes, which were mixed by inversion immediately after addition of the enzyme.  
233 Fre activity was observed as a function of the loss of NADPH, measured by re-  
234 duction of its characteristic absorbance value at  $340\text{nm}$ . Experiments were con-  
235 ducted over a period of 30 minutes at  $22^\circ\text{C}$ . All measurements were performed  
236 in triplicate.

### 237 **Luciferase purification**

238 A co-expression approach was undertaken to purify nascent LuxAB directly  
239 from *E. coli* BL21 (DE3). The luciferase genes were amplified from the plas-  
240 mid pSL1190::luxA2E bearing the *P. luminescens* luciferase operon using the  
241 high fidelity polymerase KOD (Merck-Millipore Corp.). *luxA* (Genbank ID  
242 AAA276619.1) was amplified using the primer pair:

243 luxAfw 5'-AGCACGCATATGGCGAAATTTGGAACTTTTTGCTTACA-3' /  
244 luxArv 5'-CCGTCGCTCGAGTTAATATAATAGCGAACGTTG-3'.  
245 *luxB* (Genbank ID AAA27620.1) was amplified using the primer pair:  
246 luxBfw 5'-GAGCACGCATATGGCCAAATTTGGATTGTTCTTCC-3' / luxBrv  
247 5'-CCGTCGCTCGAGTTAGGTATATTTTCATGTGGTACTTC-3'.  
248 *luxA* and *luxB* were cloned into pET21b and pET28b respectively, using a  
249 *XhoI/NcoI* digest approach. A stop codon was placed in the *luxArv* primer,  
250 leaving an N-terminally tagged LuxB and an untagged LuxA in the final ex-  
251 pression system. These plasmids were co-transformed into a version of the ex-  
252 pression strain which had previously been transformed with the plasmid pGro7,  
253 encoding the GroES/GroEL chaperone system. We found that this improved  
254 yield of soluble LuxAB. Following overnight growth of the co-expression strain in  
255 autoinduction medium containing the appropriate antibiotics and arabinose at  
256  $1\text{mgml}^{-1}$  ( $37^\circ\text{C}$  with shaking), cells were harvested by centrifugation and lysed  
257 with the Bugbuster cell lysis reagent with added benzonase and lysozyme accord-  
258 ing to the manufacturer's instructions (Merck-Millipore Corp.). Clarified lysate  
259 was diluted five-fold in wash/bind buffer (20mM tris, 200mM NaCl, pH7.4)  
260 containing protease inhibitors (Protease Inhibitor Cocktail — Sigma-Aldrich  
261 co.). The resulting preparation was applied to equilibrated  $\text{Ni}^{2+}$  sepharose  
262 columns (HiTrap - GE Healthcare Life Sciences), washed with 100 column vol-  
263 umes wash/bind buffer, and luciferase was eluted in 5 x 1ml fractions of elu-  
264 tion buffer (wash/bind buffer containing 500mM imidazole). Active fractions  
265 were assessed using the reconstituted luciferase assay (see section M4) using a  
266 Biospacelab Photon Imager, and combined. The N-terminal his-tag was cleaved  
267 from LuxB using  $3\text{ units ml}^{-1}$  cleavage-grade thrombin according to the sup-  
268 plier's instructions (Novagen). Cleaved luciferase was buffer exchanged and  
269 purified from the released tag and other low molecular weight contaminants  
270 such as imidazole using 10kDa nominal molecular weight cut-off centrifugal fil-  
271 ter units (Microcon YM10 — Sigma-Aldrich Co.). Protein concentration was  
272 determined by Bradford assay using a Nanodrop low sample volume UV-Vis  
273 Spectrophotometer (Thermo Scientific) using a BSA standard curve.

## 274 **Reconstitution of coupled luciferase assay and kinetic mea-** 275 **surements**

276 Purified luciferase and commercially prepared Fre were combined to form the  
277 coupled reaction complex as follows: Reactions were typically carried out in

278 a final volume of 100 $\mu$ l containing purified luciferase (typically in the range  
279 1.5-15 $\mu$ gml<sup>-1</sup>), NADPH (200 $\mu$ M), FMN (tested over the range 10nM-100 $\mu$ M  
280 in this study), Fre (0.2 units ml<sup>-1</sup>) and decanal (0.02%) in 50mM potassium  
281 phosphate buffer, pH 7.0. Reactions were carried out at 22°C and monitored in  
282 either a Biospacelab Photon Imager or Tecan Genios Pro multimode microplate  
283 reader. The kinetic assays were carried out using a Tecan Genios pro fitted with  
284 injector capacity as follows; all components other than decanal were combined  
285 in a final volume of 50 $\mu$ l per well in flat bottomed microtiter plates suitable  
286 for bioluminescence measurements. The reactions were initiated by injection  
287 of a further 50 $\mu$ l phosphate buffer containing 0.04% decanal. Reactions were  
288 monitored for 1100 milliseconds. All measurements were performed in triplicate.

## 289 Estimation of Lux Turnover Rates

290 The promoter of the Universal hexose transporter (*uhpT*) gene was amplified  
291 from *S. aureus* and introduced into pUNK1dest along with the Gram-positive  
292 GFP-*lux*ABCDE operon and the *rrn*BT1T2 terminator [24] using a Multisite  
293 Gateway LR plus reaction. Transformants were selected on erythromycin and  
294 screened for expression of the reporter. This PuhpT-reporter vector was des-  
295 ignated pSB3009. *S. aureus* RN4220 [pSB3009] overnight cultures were grown  
296 aerobically at 37°C in in Tris Minimal Succinate medium (TMS, [25]) supple-  
297 mented with Erythromycin (5 $\mu$ gml<sup>-1</sup>) for plasmid maintenance Bacterial pellets  
298 were washed once in TMS without sodium succinate (TM) and resuspended in  
299 an equal volume of TM. These were diluted 1/50 into fresh TM supplemented  
300 with filter sterilized sugars (Glucose or glucose-6-phosphate) supplemented with  
301 Erythromycin (5 $\mu$ gml<sup>-1</sup>).

302 For growth and reporter gene measurements, replicate samples (200 $\mu$ l) were  
303 placed into the wells of a 96-clear-bottom microtiter plate (Porvair) and in-  
304 cubated at 37°C in a Tecan Genesis Pro microplate reader. Optical density  
305 (600nm), fluorescence (485ex/510em) and bioluminescence (RLU) readings were  
306 taken at 30 min periods over the course of the experiment.

307 From the data generated from these experiments, we identified the curves  
308 where RLU (light read-outs, arbitrary units) decreases while cells are still in  
309 exponential phase (corresponding OD measurements). We estimate the turn-  
310 over rate for each such curve (total 18 curves used) by fitting a linear line to the  
311 log transformed data. The histogram of estimated values are shown in Figure  
312 1d. The linear fits to the log-transformed data for all the 18 curves is shown in

313 Supplementary Material.

## 314 **Parameter Inference**

315 In order to estimate the kinetic parameters for all three reactions, we used a  
316 similar Bayesian approach based on Markov Chain Monte Carlo (MCMC), in  
317 which a sub-model relevant to each reaction is used along-with corresponding  
318 experimental data. We used an adaptive version of Metropolis-Hasting MCMC  
319 algorithm with global scaling[26] in order to iteratively sample from the poste-  
320 rior distribution of the kinetic parameters. Our choice of priors and likelihood  
321 function is described below:

322 Unless otherwise specified, we used un-informative exponential priors ( $\lambda =$   
323 100) for kinetic parameters, while more informative *gamma* priors ( $Gam(0.9, 0.1)$ )  
324 for degradation rates. This reflects our *a priori* knowledge that the kinetic pa-  
325 rameters (ratio of kinetic rate constants) are all positive real numbers. For those  
326 parameters which are common to the inference in both the Fre and LuxAB mod-  
327 els, prior estimates for the LuxAB inference were generated by fitting exponen-  
328 tial distributions to the posterior estimates derived from the the Fre inference.

329 We define the likelihood of the parameters, for any of the models using in  
330 equation 1, assuming homogeneous Gaussian noise. Given the current set of  
331 parameter values ( $\theta$ ), we simulate the species of interest (stored in vector  $Y'$ ),  
332 and use the corresponding experimental data in vector  $Y$  to give the likelihood  
333 function:

$$L(\theta) = \prod_{i=1}^n \left( \frac{\tau}{2\pi} \right)^{\frac{1}{2}} \exp\left(-\frac{\tau}{2}(Y_i - Y'_i)^2\right) \quad (1)$$

334 A separate Gibbs step is introduced for the sampling of noise precision  $\tau$ ,  
335 in case of Fre and LuxEC inference, while for LuxAB data, we estimated the  
336 noise variance from the replicates. Details on the derivation of the Gibbs step  
337 is provided in supplementary methods.

## 338 **Promoter Inference**

339 A Monte Carlo approach was used to infer promoter activity from light readout.  
340 The promoter input function is modelled as a series of K heights at fixed posi-  
341 tions. A Martingale prior distribution is used[27], so that the prior distribution  
342 for each height at point  $n$  is an exponential distribution with mean value equal

343 to the current height at the previous point  $n - 1$ . At each step, a point is chosen  
344 at random, and a new height is proposed, as described in Green 1995[28]. The  
345 likelihood function uses a Gaussian error model. For all promoter inference,  
346 light output curves for a whole experiment must be normalized to the highest  
347 light value found in that experiment.

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## 351 Competing interests

352 The authors declare that they have no competing financial interests.

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## 356 References

- 357 [1] Meighan, E. Genetics of bacterial bioluminescence. *Annu Rev Genet.* **28**,  
358 117–139 (1994).
- 359 [2] Dunlap, P. Biochemistry and genetics of bacterial bioluminescence. *Ad-  
360 vances in Biochemical Engineering and Biotechnology* **144**, 37–64 (2014).
- 361 [3] Szittner, R. & Meighan, E. Nucleotide sequence, expression, and properties  
362 of luciferase coded by *lux* genes from a terrestrial bacterium. *The Journal  
363 of Biological Chemistry* **265**, 16581–16587 (1990).
- 364 [4] Close, D. *et al.* The evolution of the bacterial luciferase gene cassette (*lux*)  
365 as a real-time bioreporter. *Sensors* **12**, 732–752 (2012).
- 366 [5] Qazi, S., Harrison, S., Self, T., Williams, P. & Hill, P. Real-time monitoring  
367 of intracellular *Staphylococcus aureus* replication. *Journal of Bacteriology*  
368 **186**, 1065–1077 (2004).
- 369 [6] Zaslaver, A. *et al.* Just-in-time transcription program in metabolic path-  
370 ways. *Nature Genetics* **36**, 486–491 (2004).

- 371 [7] Close, D. *et al.* Autonomous bioluminescent expression of the bacterial  
372 luciferase gene cassette (lux) in a mammalian cell line. *PLoS One* **5**, e12441  
373 (2010).
- 374 [8] Brutescio, C. *et al.* Novel aspects of the acid response network of *Escherichia*  
375 *coli K-12* are revealed by a study of transcriptional dynamics. *Environ Sci*  
376 *Pollut Res* **24**, 52–65 (2017).
- 377 [9] Bacconi, M. *et al.* A stable luciferase reporter plasmid for in vivo imag-  
378 ing in murine models of staphylococcus aureus infections. *Appl Microbial*  
379 *Biotechnol* **100**, 3197–3206 (2016).
- 380 [10] Shivak, D. *et al.* A modular, tn7-based system for making bioluminescent or  
381 fluorescent salmonella and escherichia coli strains. *Appl Environ Microbiol*  
382 **82**, 4931–4943 (2016).
- 383 [11] Cronin, M., Akin, A., Francis, K. & Tangney, M. In vivo bioluminescence  
384 imaging of intratumoral bacteria. *Methods Mol Biol* **1409**, 69–77 (2016).
- 385 [12] King, J. *et al.* Rapid, sensitive bioluminescent reporter technology for  
386 naphthalene exposure and biodegradation. *Science* **249**, 778–781 (1990).
- 387 [13] Zanzotto, A. *et al.* In situ measurement of bioluminescence and fluorescence  
388 in an integrated microbioreactor. *Biotechnology and Bioengineering* **93**,  
389 40–47 (2006).
- 390 [14] Qazi, S. *et al.* N-acylhomoserine lactones antagonize virulence gene expres-  
391 sion and quorum sensing in *Staphylococcus aureus*. *Infection and Immunity*  
392 **74**, 910–919 (2006).
- 393 [15] Burton, N., Johnson, M., Antczak, P., Robinson, A. & Lund, P. Novel  
394 aspects of the acid response network of *Escherichia coli K-12* are revealed  
395 by a study of transcriptional dynamics. *Journal of Molecular Biology* **401**,  
396 726–742 (2010).
- 397 [16] Jia, K., Eltzov, E., Marks, R. & Ionescu, R. Bioluminescence enhancement  
398 through an added washing protocol enabling a greater sensitivity to carbo-  
399 furan toxicity. *Ecotoxicology and Environmental Safety* **96**, 61–66 (2013).
- 400 [17] Welham, P. & Stekel, D. Mathematical model of the lux luminescence sys-  
401 tem in the terrestrial bacterium *photorhabdus luminescens*. *Mol. BioSyst.*  
402 **5**, 68–76 (2009).

- 403 [18] Campbell, Z., Weichsel, A., Montfort, W. & Baldwin, T. Crystal structure  
404 of the bacterial luciferase/flavin complex provides insight into the function  
405 of the beta subunit. *Biochemistry* **48**, 6085–6094 (2009).
- 406 [19] King, E. & Altman, C. A schematic method of deriving the rate laws for  
407 enzyme-catalyzed reactions. *J. Phys. Chem.* **60**, 1375–1378 (1956).
- 408 [20] Takahashi, H. *et al.* The dynamic balance of import and export of zinc  
409 in *Escherichia coli* suggests a heterogeneous population response to stress.  
410 *Journal of the Royal Society Interface* **12**, DOI:10.1098/rsif.2015.0069  
411 (2015).
- 412 [21] Fletcher, S., Iqbal, M., Jabbari, S., Stekel, D. & Rappoport, J. Analysis of  
413 occludin trafficking, demonstrating continuous endocytosis, degradation,  
414 recycling and biosynthetic secretory trafficking. *PLoS ONE* **9**, e111176  
415 (2014).
- 416 [22] Rodriguez, A., Riendeau, D. & Meighan, E. Purification of the acyl coen-  
417 zyme a reductase component from a complex responsible for the reduction  
418 of fatty acids in bioluminescent bacteria. properties and acyltransferase  
419 activity. *The Journal of Biological Chemistry* **258**, 233–5237 (1983).
- 420 [23] Rodriguez, A., Nabi, I. & Meighan, E. Atp turnover by the fatty acid  
421 reductase complex of *Photobacterium phosphoreum*. *Canadian Journal of*  
422 *Biochemistry and Cell Biology* **63**, 1106–1111 (1985).
- 423 [24] Pehinec, T. *et al.* Construction and evaluation of multisite recombinato-  
424 rial (gateway) cloning vectors for gram-positive bacteria. *BMC Molecular*  
425 *Biology* **8**, 80 (2007).
- 426 [25] Sebulsky, M., Hohnstein, D., Hunter, M. & DE, H. Identification and  
427 characterization of a membrane permease involved in iron-hydroxamate  
428 transport in *Staphylococcus aureus*. *Journal of Bacteriology* **182**, 4394–  
429 4400 (2000).
- 430 [26] Andrieu, C. & Thoms, J. A tutorial on adaptive mcmc. *Statistics and*  
431 *Computing* **18**, 343–373 (2008).
- 432 [27] Arjas, E. & Gasbarra, D. Nonparametric bayesian inference from right  
433 censored survival data, using the gibbs sampler. *Statistica sinica* **4**, 505–  
434 524 (1994).

- 435 [28] Green, P. Reversible jump markov chain monte carlo computation and  
436 bayesian model determination. *Biometrika* **82**, 711–732 (1995).