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A simple biosynthetic pathway for large product generation from small substrate amounts

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Abstract
A recently emerging discipline of synthetic biology has the aim of constructing new biosynthetic pathways with useful biological functions. A major application of these pathways is generating a large amount of the desired product. However, toxicity due to the possible presence of toxic precursors is one of the main problems for such production. We consider here the problem of generating a large amount of product from a potentially toxic substrate. To address this, we propose a simple biosynthetic pathway, which can be induced in order to produce a large number of the product molecules, by keeping the substrate amount at low levels. Surprisingly, we show that the large product generation crucially depends on fast non-specific degradation of the substrate molecules. We derive an optimal induction strategy, which allows as much as three orders of magnitude increase in the product amount through biologically realistic parameter values. We point to a recently discovered bacterial immune system (CRISPR/Cas in *E. coli*) as a putative example of the pathway analysed here. We also argue that the scheme proposed here can be used not only as a stand-alone pathway, but also as a strategy to produce a large amount of the desired molecules with small perturbations of endogenous biosynthetic pathways.

1. Introduction

Synthetic biology is an emerging scientific discipline, which aims to design novel biological circuits for desired applications [1]. There has recently been a great deal of progress (e.g. in DNA synthesis and sequence manipulation) that enhances the construction of these new pathways [2, 3]. However, the development of these technological tools has outpaced our understanding of the fundamental design principles for the construction of these circuits [4].

A major application for synthetically designed pathways is to produce a large amount of the desired molecules. However, one of the main obstacles in such production is the toxicity due to the possible presence of toxic precursors [4–6]. Consequently, we consider here the problem of how to keep the substrate (that can be toxic above some level) at small amounts, while producing a large amount of useful molecules upon system induction.

To address this problem, we consider a simple biosynthetic pathway, which is represented by the set of biochemical reactions shown in figure 1. This reaction set defines a process where the substrate s is generated with rate ϕ, degraded with rate λ_s and processed to product p with rate k. The product is further degraded with rate λ_p. We, furthermore, assume that both ϕ and k are subject to regulation, i.e. can be increased when the system is induced (when a large amount of the product needs to be generated). The decay rates (λ_s and λ_p) are constants that characterize the system.

Based on the discussion above, our goal is to find an optimal strategy for activation of the system in figure 1. Specifically, this strategy should satisfy the following: (i) there is a large relative increase in the product amount, upon the system induction, (ii) the amount of the substrate $[s]$ does not increase (remains low), when the system is induced, (iii) the initial (uninduced) substrate amount should be comparable to the product amount. The last condition provides that the initial

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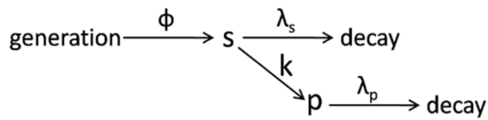

Figure 1. Proposed biosynthetic pathway. s and p correspond, respectively, to a substrate and a product. The substrate s and the product p decay, respectively, with rates λ_s and λ_p. The substrate s is generated with rate ϕ and converted to the product with rate k.

amount of (potentially toxic) substrate is low. In addition to these basic conditions, we also aim that the system induction is achieved with as small a change in the parameter values as feasible.

In this paper, we will show that the simple system presented by figure 1 is capable of producing the behaviour defined above. We will also derive a realistic induction strategy, for which the above conditions are optimally satisfied. We will furthermore show that such an induction strategy can lead to a very large (three orders of magnitude) increase in the product amount, for realistic parameter values. Surprisingly, a crucial ingredient of this large increase is a loss of substrate through fast non-specific degradation. Finally, we will discuss biological applications of the mechanism analysed here.

2. Results and discussion

2.1. Analytical results

In this subsection we analytically study induction of the system defined by the reaction scheme in figure 1. It is useful to observe the following three cases: (i) increasing the substrate to product processing rate (k), while keeping the substrate production rate (ϕ) constant; (ii) increasing ϕ while keeping k constant and (iii) increasing both ϕ and k. It is straightforward to see that an increase of k (the first case) leads to a decrease of the substrate and an increase of the product. On the other hand, an increase of ϕ (the second case) leads to an increase of both the substrate and the product. Finally, the third case (an increase of both k and ϕ) is a mix of the previous two cases; therefore, through an appropriate balance between the increase of k and ϕ, one can obtain a larger amount of product, without increasing the substrate amount. In the analysis below, we will concentrate on this case (the increase of both k and ϕ) and infer an optimal induction strategy through which one can obtain a large product amount, while keeping the substrate amount at low levels.

Note that processing of the substrate to product in figure 1 involves an enzyme catalysis and should, in principle, be presented by the Michaelis–Menten law. In the limit of the small substrate concentrations (i.e. when the substrate concentration is much smaller than the Michaelis–Menten constant (K_m)), the substrate to product processing reduces to first order kinetics. Since we here impose that the (potentially toxic) substrate has to be kept at low concentrations, we model the mechanism in figure 1 by first order kinetics; e.g. note that ~10 substrate molecules corresponds to ~10nM substrate concentration for bacterial cell, which is much smaller than the typical values of Michaelis–Menten constants. However, at the end of this subsection, we will analyse the mechanism through Michaelis–Menten law, in order to account for the possibility of very strong enzyme binding (low K_m), and show that the optimization strategy is robust with respect to the models/approximations used.

Equations that determine the kinetics of the system, upon its induction, are given in the appendix. For the first order kinetics, it is straightforward to show that the relative change in the steady-state product and substrate amounts is given by (see appendix A):

$$\frac{[p]'}{[p]} = \frac{\frac{\lambda_s}{k}+1}{\frac{\lambda_s}{k'}+1}\frac{\phi'}{\phi}, \quad \frac{[s]'}{[s]} = \frac{\lambda_s+k}{\lambda_s+k'}\frac{\phi'}{\phi}. \tag{1}$$

The terms in equation (1) are defined in the previous section and in figure 1. The 'primes' correspond to the steady state values upon the system induction; e.g. k and k' denote, respectively, the substrate processing rates before and after the system induction.

Note that the properties of the system, qualitatively discussed above, can be directly inferred from equation (1). Furthermore, if k' becomes much larger than λ_s, a further increase in k' does not lead to an additional relative increase in the product amount (i.e. $[p]'/[p] \to \lambda_s/k+1$, for $k' \gg \lambda_s$). Such saturation arises since, when $k' \gg \lambda_s$, almost all of the generated substrate is processed to the product, and therefore a further increase in k' does not have an additional effect on the product gain.

Next we consider an optimal method for the system induction through the joint increase of ϕ and k; note that such a method has to satisfy the three conditions stated in the introduction. From equation (1) it follows that the condition $[s]'/[s] \leqslant 1$ leads to

$$\frac{\phi'}{\phi} \leqslant \frac{\lambda_s+k'}{\lambda_s+k}. \tag{2}$$

Since $[p]'/[p]$ is directly proportional to ϕ'/ϕ (see equation (1)), in order to achieve the maximal $[p]'/[p]$, one must use the maximal ϕ'/ϕ, that still satisfies equation (2). Such a value of ϕ'/ϕ, leads to a simple expression for the maximal value of the product increase $([p]'/[p])_{\max} = k'/k$. That is, no matter how we change ϕ and k, the maximal value of the product increase is determined by only the relative increase in the processing rate, as long as the substrate amount does not increase. However, note that only increasing the processing rate is not sufficient to achieve this maximal value, i.e. the production rate also has to be increased according to the maximal value allowed by equation (2).

Therefore, the optimal induction strategy requires an as large as possible relative increase in the processing rate k'/k. However, the ratio of k'/k is, in reality, constrained from above, i.e. it is determined by the maximal relative increase in the amount of the enzyme that catalyses processing of the substrate to the product. Consequently, for an optimal system induction, one should (i) increase k'/k as much as is realistically possible, and (ii) for such k' value, increase ϕ'/ϕ for the maximal value that is allowed by equation (2). This will result in a relative increase of the steady state product amount that is equal to k'/k, and in an absence of an increase of the substrate amount.

Next we investigate how changes in the substrate and the product stability influence product generation. The condition that an initial (steady state) substrate amount has to be comparable to the initial product amount, leads to $k \sim \lambda_p$ (see appendix A). Furthermore, if we label the maximal relative increase in the transcript processing rate as $(k'/k)_{\max} = M$, from equation (2) and from $k \sim \lambda_p$, we obtain that the relative increase of the substrate generation rate, which leads to the optimal (maximal) product generation, is approximately given by

$$\frac{\phi'}{\phi} \sim 1 + \frac{M-1}{\frac{\lambda_s}{\lambda_p}+1}. \tag{3}$$

From the above equation, we see that a slower substrate decay λ_s requires a larger relative increase in the substrate generation rate. Due to this, we obtain an unexpected result that a larger (nonspecific) loss of the substrate leads to a more efficient system induction.

Next we consider how the analysis changes when the substrate to product processing is modelled by the Michaelis–Menten law. In appendix B, we show that the strategy for the optimal system induction remains unchanged when the Michaelis–Menten law is used. That is, the maximal value of the relative product increase is still determined by the increase in the substrate processing rate $([p]'/[p])_{\max} = k'/k$. Since a large increase in the processing rate is required for large product generation, we further assume that k' reaches the saturation regime (i.e. $k' \gg \lambda_s$).

We next derive the extent of the relative ϕ increase, which is necessary to achieve the maximal product increase (i.e. the equivalent of the equation (2) for the Michaelis–Menten law). In the derivation, we assume that $\lambda_s \gg k$, since we previously obtained that a large product increase requires $\lambda_s \gg \lambda_p \sim k$. Consequently, under approximations $k' \gg \lambda_s \gg k$, we obtain (see appendix B):

$$\frac{\phi'}{\phi} \approx 1 + \frac{k'}{\lambda_s}\frac{1}{s/K_m+1}. \tag{4}$$

Note that, under the same approximations, equation (2) reduces to $\phi'/\phi \approx k'/\lambda_s$. Therefore, Michaelis–Menten law requires a smaller increase of the production rate necessary to obtain the maximal product gain, i.e. leads to an even more efficient induction. Consequently, the derived induction strategy is robust with respect to the used model/assumptions.

2.2. Numerical analysis

In this subsection, we will numerically investigate the induction of the system represented by figure 1. We will use first order kinetics in the numerical analysis, since it corresponds to the relevant physical limit (low substrate concentration), and since we have shown that using the Michaelis–Menten law does not affect the induction strategy. We will simulate the system both deterministically and stochastically[3]; this will allow us to also visualize intrinsic fluctuations for the small number of the substrate molecules. We will first analyse the case of fast substrate and slow product decay ($\lambda_s = 1$ min^{-1} and $\lambda_p = 1/100$ min^{-1}), and then investigate the effects of increasing the substrate stability.

In figure 2, we analyse how the product amount $[p]$ increases when only the processing rate k is increased. Consequently, we consider an increase of k for 10 times (figure 2(*A*)), 100 times (figure 2(*B*)), 1000 times (figure 2(*C*)) and 10 000 times (figure 2(*D*))[4]. We see that, as k reaches 1000, the steady state value of the generated product reaches saturation, i.e. stops increasing with a further increase in k. We note that this observation is in accordance with the analysis presented in the previous subsection; that is (i) saturation is achieved when $k' \gg \lambda_s$, which corresponds to $k'/k \sim 1000$ for the numerical values used here, and (ii) the saturation level (i.e. maximum) for the relative product increase is approximately equal to λ_s/k (i.e. $[p]'/[p] \sim 100$, for the numerical values used here). Therefore, in order to relieve such saturation, the production rate has to be increased as well.

The joint increase of k and ϕ is analysed in figure 3. For both panels, k is increased 1000 times, which corresponds to the value for which saturation in the steady state amount of the product $[p]$ is observed. In figure 3(*A*), ϕ is increased ten times, which leads to the desired behaviour of the system:

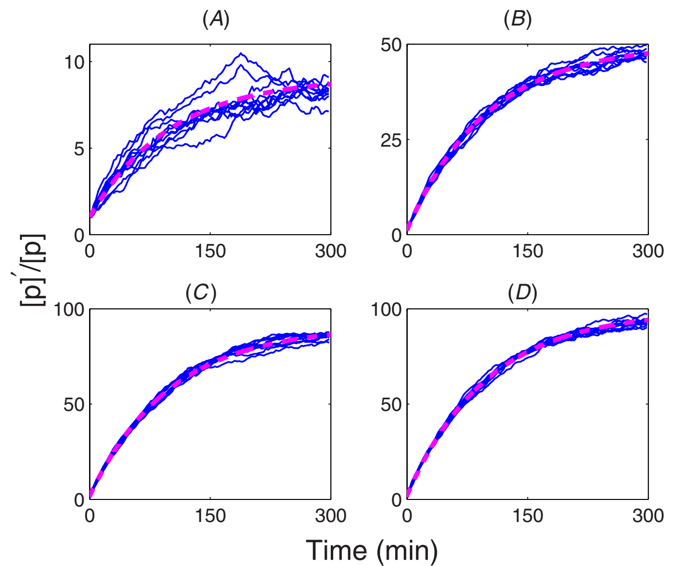

Figure 2. Saturation in the product amounts. The reaction scheme in figure 1 is simulated both deterministically (magenta dashed line) and stochastically (full blue curves) through the Gillespie stochastic simulation algorithm; ten stochastic trajectories are shown. The substrate and the product decay rates are respectively, $\lambda_s = 1$ and $\lambda_p = 1/100$ min^{-1}. Initial substrate generation and processing rates (ϕ and k) are adjusted so that the (deterministic) steady state values of the substrate and the product are $[s] = 20$, $[p] = 20$. We then increase the substrate processing rate for (*A*) 10 times, (*B*) 100 times, (*C*) 1000 times and (*D*) 10 000 times. The figure shows that an increase of only k can lead to a large (two orders of magnitude) increase of the product amount; however, the product level stops increasing when k becomes sufficiently large (which we call saturation).

[3] Note that stochastic simulations can lead to a significantly larger estimate of the substrate amounts, when biomolecular interactions are taken into account, and when the enzyme is saturated [7]. We do not analyse such a possibility here, since the relevant regime is the one in which the substrate (rather than the enzyme) is saturated.

[4] Note that the stochastic and the deterministic means in figures 2–4 correspond to each other, as expected [8, 9].

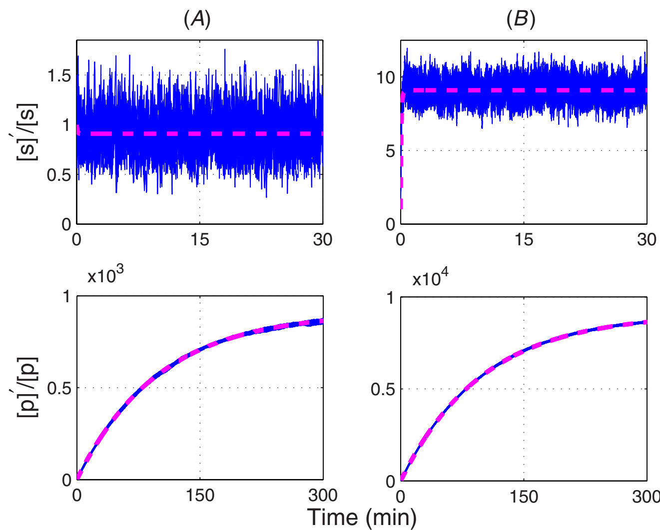

Figure 3. Abolishing saturation in the product amount. The decay rates (λ_s and λ_p) and the initial conditions are the same as in figure 2. In both (*A*) and (*B*), the substrate processing rate k is increased 1000 times, while the substrate generation rate ϕ is (*A*) increased 10 times and (*B*) increased 100 times. The figure shows that saturation, which is exhibited when only k is increased, is relieved when k and ϕ are both increased; however, too large an increase in ϕ leads to an (undesired) increase in the substrate amounts.

saturation of the product $[p]$, shown in figure 2(*C*) is abolished (i.e. the amount of the product is increased for an additional order of magnitude), while the amount of the substrate $[s]$ does not increase. Note that, for the numerical values used here, this increase in ϕ approximately corresponds to the optimal value ($(\phi'/\phi)_{\max} \sim k'/\lambda_s$—see section 2.1). Consequently, the relative increase of the steady state product amount (approximately 1000) becomes equal to the maximal value ($k'/k = 1000$).

In figure 3(*B*), ϕ is increased for an additional order of magnitude (100 times), which however leads to a substantial increase in the substrate amount $[s]$. Such an increase is a consequence of the fact that equation (2) is no longer valid. We therefore conclude that (for the numerical values used here) the optimal strategy for a large product gain, that does not result in the substrate increase, is a joint 1000-fold increase of k and 10-fold increase in ϕ; this strategy leads to the product increasing by k'/k (in our case 1000 times).

One should note that the parameters leading to the three orders in magnitude increase of the product amount in figure 3(*A*) are biologically realistic: $\lambda_s = 1$ and $\lambda_p = 1/100\ \text{min}^{-1}$ are well within the degradation rates for both proteins and transcripts, whose half lives are in a range of minutes to hours/days. A three orders in magnitude increase in the processing rate k may also be achieved, since it is within a realistic range of the activation of a promoter transcribing the enzyme gene. Finally, it is evident that an order of magnitude increase in the substrate generation rate can also be achieved.

Moreover, a simple mechanism in figure 1 matches with a current minimal model of transcript processing [13] in a recently discovered *E. coli* immune system CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR associated sequences) [10–12]. Another

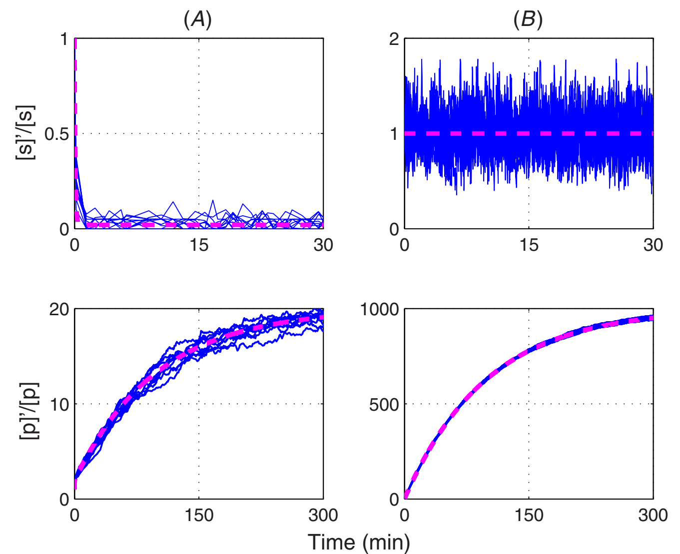

Figure 4. Change of substrate stability. The panel arrangement, the initial substrate and product amounts, and the product stability are the same as in figure 3. The substrate stability is increased, so that it becomes equal to the product stability (i.e. $\lambda_s = \lambda_p = 1/100\ \text{min}^{-1}$). (*A*) The increase in k and ϕ is the same as in figure 3(*A*) (k is increased 1000 times and ϕ 10 times), (*B*) ϕ is now increased for an additional two orders of magnitude (i.e. both k and ϕ are increased 1000 times). The figure shows that, if the substrate stability is increased, the increase in the parameter values has to be much larger in order to achieve the same product gain.

argument that the parameters in figure 3(*A*) are biologically realistic, is that they also roughly match those of the *E. coli* CRISR/Cas system. That is, the half lives of pre-crRNA (substrate) and crRNA (product) are, respectively, on the order of minutes and hours [13]; furthermore, during (artificial) system induction the processing rate of the enzyme that catalysis pre-crRNA to crRNA processing, is increased for about three orders of magnitude [13], which corresponds to the magnitude of k increase in figure 3(*A*).

Next we consider the extent of the intrinsic fluctuations in the steady state substrate amounts, for both the uninduced system and for the optimal induction parameters (figure 3(*A*)); for a toxic substrate, it may be important that these fluctuations are reasonably low. To obtain sufficiently large statistics, we perform 1000 stochastic simulations for both the uninduced and the optimally induced system, and record the number of molecules once the steady states are established. The obtained 95% confidence limits correspond to (20 ± 9) molecules for the uninduced system, and (19 ± 8) molecules for the optimal induction. We therefore conclude that intrinsic substrate fluctuations are kept at reasonably low levels—both before and after the induction—which is desirable for the case when the substrate molecules are poisonous above a certain threshold.

In the analysis above, we showed that fast substrate decay ($\lambda_s = 1\ \text{min}^{-1}$) and slow product decay ($\lambda_p = 1/100\ \text{min}^{-1}$) lead to the desired system behaviour (a large product increase). In figure 4, we analyse what happens if the substrate stability is increased, so that it becomes equal to the product stability ($\lambda_s = \lambda_p = 1/100\ \text{min}^{-1}$). In figure 4(*A*), we increase k and

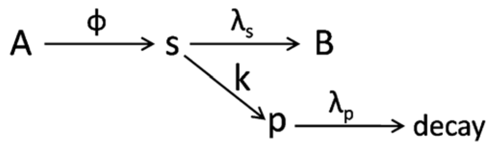

Figure 5. Modifying endogenous biosynthetic pathway. The substrate is generated from species A with rate ϕ, and processed to species B with rate λ_s (the notation is used to facilitate a comparison with figure 1). The substrate s, and species A and B are part of an endogenous biosynthetic pathway. As a synthetic addition to the pathway, product p (with decay rate λ_p) is processed from substrate $[s]$ with rate k.

ϕ for the same amount as in figure 3(*A*) (k for 1000 fold and ϕ for 10 fold), but observe a much smaller increase in the steady state product amount, compared to the case in figure 3(*A*). In figure 4(*B*), we increase both k and ϕ 1000 fold (in accordance with the optimal induction for such a substrate stability), which then leads to the same product increase as in figure 3(*A*). Therefore, we obtain a counter-intuitive result that, in the case of larger substrate stability, one needs a much larger increase in the substrate production rate ϕ to achieve the same (optimal) product increase. This result is consistent with the analytical results in the previous subsection.

2.3. Modifying endogenous biosynthetic pathways

We here analysed the problem from the perspective of the traditional 'from scratch' approach to synthetic biology, where pathways are designed to function as independently as possible from the underlying cellular systems. On the other hand, there has recently been an approach where synthetic gene circuits are closely integrated with endogenous processes. Within such an approach, it is ideal to minimally perturb an endogenous pathway, while still producing the desired outcome [1].

It is straightforward to see that the system defined here naturally fits within such an approach, as indicated by figure 5. The substrate s can be a part of any biosynthetic pathway, as long as it is efficiently converted to the downstream chemical ((*B*) in figure 5). That is, the unspecific decay rate of the substrate λ_s in our analysis (which should be sufficiently large) takes the role of the rate of conversion to the downstream chemical. Similarly, the flux through the upstream part of the pathway ((*A*) in figure 5), takes the role of the substrate production rate ϕ in figure 1.

Processing of the substrate to the desired product with rate k can then be included as a synthetic 'add-on' to the endogenous biosynthetic pathway. Similarly, as in figure 1, the rates k and ϕ have to be regulated in order to allow induction of the product synthesis. The optimal induction strategy derived here allows for increasing the product to a very large amount, with a much smaller increase of the flux through the upstream part of the pathway. Furthermore, we showed that the optimal induction strategy corresponds to the case when the substrate amount is unchanged, which allows the generation of large product amounts without perturbing the downstream reactions in the endogenous pathway. Therefore, we expect that the system analysed here will find a large number of applications in the future, as either a stand alone biosynthetic pathway, or as a synthetic modification of an already existing endogenous pathway.

3. Conclusion and outlook

We proposed here a simple mechanism which allows a large (three orders of magnitude) increase of the product amount, without increasing the substrate amount. Surprisingly, a crucial element of this large increase is a fast non-specific degradation of the substrate molecules; this establishes that non-specific loss of substrate may, counter-intuitively, enhance large product generation. We pointed to the CRISPR/Cas system in *E. coli*, as a putative example of the pathway proposed here. Interestingly, the parameters optimized here roughly match those measured for the CRISPR/Cas system. The significance of this finding has yet to be understood, since the mechanism of the natural CRISPR/Cas system induction is the focus of current research. We finally pointed out that the scheme employed here can be used not only as a stand-alone pathway, but also as a synthetic modification of an endogenous pathway. We therefore expect that the simple system analysed here will provide understanding necessary for both analysing and modifying the existing, and constructing novel biosynthetic pathways.

Acknowledgments

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Appendix A. Kinetic equations—first order kinetics

By using the first order kinetics, we obtain the kinetic equations that describe the set of reactions shown in figure 1:

$$\frac{d[s]}{dt} = \phi - \lambda_s[s] - k[s] \quad \text{(A.1)}$$

$$\frac{d[p]}{dt} = -\lambda_p[p] + k[s]. \quad \text{(A.2)}$$

The notation used in the above equations is the same as in figure 1. In steady state we have that $d[s]/dt = 0$ and $d[p]/dt = 0$, so:

$$0 = \phi - \lambda_s[s] - k[s] \quad \text{(A.3)}$$

$$0 = -\lambda_p[p] + k[s]. \quad \text{(A.4)}$$

When the system is induced (k and ϕ are increased to k' and ϕ'), the equations become:

$$0 = \phi' - \lambda_s[s]' - k'[s]' \quad \text{(A.5)}$$

$$0 = -\lambda_p[p]' + k'[s]'. \quad \text{(A.6)}$$

To derive equation (1) in section 2, we express $[p]$ from equations (A.3) and (A.4), $[p]'$ from equations (A.5) and (A.6), $[s]$ from equation (A.3) and $[s]'$ from equation (A.5). To derive equation (2), we use that $[s]'/[s]$, given by equation (1) has to be smaller than 1.

Appendix B. Kinetic equations—Michaelis–Menten law

By using the Michaelis–Menten law, the kinetic equations that describe the set of reactions shown in figure 1 become (K_m is Michaelis–Menten constant):

$$\frac{\mathrm{d}[s]}{\mathrm{d}t} = \phi - \lambda_s[s] - k\frac{[s]}{[s]/K_m + 1} \quad \text{(B.1)}$$

$$\frac{\mathrm{d}[p]}{\mathrm{d}t} = -\lambda_p[p] + k\frac{[s]}{[s]/K_m + 1}. \quad \text{(B.2)}$$

In steady state, $\mathrm{d}[s]/\mathrm{d}t = 0$ and $\mathrm{d}[p]/\mathrm{d}t = 0$, leading to:

$$0 = \phi - \lambda_s[s] - k\frac{[s]}{[s]/K_m + 1} \quad \text{(B.3)}$$

$$0 = -\lambda_p[p] + k\frac{[s]}{[s]/K_m + 1}. \quad \text{(B.4)}$$

When the system is induced (k and ϕ are increased to k' and ϕ'), the equations become:

$$0 = \phi' - \lambda_s[s]' - k'\frac{[s]'}{[s]'/K_m + 1} \quad \text{(B.5)}$$

$$0 = -\lambda_p[p]' + k'\frac{[s]'}{[s]'/K_m + 1}. \quad \text{(B.6)}$$

By using equations (B.4) and (B.6), we obtain:

$$\frac{[p]'}{[p]} = \frac{k'}{k}\frac{[s]'}{[s]}\frac{[s]/K_m + 1}{[s]'/K_m + 1}. \quad \text{(B.7)}$$

Note that $[p]'/[p]$ increases with the increase in $[s]'/[s]$. It is therefore evident that the maximal relative increase in the product (without increasing the substrate) is achieved when $[s]' = [s]$, leading to $([p]'/[p])_{\max} = k'/k$.

We next derive for how much ϕ needs to increase to achieve a maximal increase in the product, without increasing the substrate. By using the approximation $k \ll \lambda_s \ll k'$ (see section 2.1), from equation (B.3) it is straightforward to obtain $[s] \approx \phi/\lambda_s$. Since the maximal increase in the product is achieved when the substrate remains unchanged, we substitute this value into equation (B.5) to obtain

$$\frac{\phi'}{\phi} \approx 1 + \frac{k'}{\lambda_s}\frac{1}{[s]/K_m + 1}. \quad \text{(B.8)}$$

References

[1] Nandagopal N and Elowitz M B 2011 Synthetic biology: integrated gene circuits *Science* **333** 1244

[2] Gibson D G *et al* 2010 Creation of a bacterial cell controlled by a chemically synthesized genome *Science* **329** 52

[3] Wang H H, Isaacs F J, Carr P A, Sun Z Z, Xu G, Forest C R and Church G M 2009 Programming cells by multiplex genome engineering and accelerated evolution *Nature* **460** 894

[4] Weeks A M and Chang M C 2011 Constructing de novo biosynthetic pathways for chemical synthesis inside living cells *Biochemistry* **50** 5404

[5] Jones K L, Kim S W and Keasling JD 2000 Low-copy plasmids can perform as well as or better than high-copy plasmids for metabolic engineering of bacteria *Metab. Eng.* **2** 328

[6] Pitera D J, Paddon C J, Newman J D and Keasling J D 2007 Balancing a heterologous mevalonate pathway for improved isoprenoid production in *Escherichia coli* *Metab. Eng.* **9** 193

[7] Grima R 2009 Investigating the robustness of the classical enzyme kinetic equations in small intracellular compartments *BMC Syst. Biol.* **3** 101

[8] Gadgila C, Leea C H and Othmerb H G 2005 A stochastic analysis of first-order reaction networks *Bull. Math. Biol.* **67** 901

[9] Heuett W J and Qian H 2006 Grand canonical Markov model: a stochastic theory for open nonequilibrium biochemical networks *J. Chem. Phys.* **124** 044110

[10] Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero D A and Horvath P 2007 CRISPR provides acquired resistance against viruses in prokaryotes *Science* **315** 1709

[11] Makarova K S, Grishin N V, Shabalina S A, Wolf Y I and Koonin E V 2006 A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action *Biol. Direct* **1** 7

[12] Al-Attar S, Westra E R, van der Oost J and Brouns S J 2011 Clustered regularly interspaced short palindromic repeats (CRISPRs): the hallmark of an ingenious antiviral defense mechanism in prokaryotes *Biol. Chem.* **392** 277

[13] Pougach K, Semenova E, Bogdanova E, Datsenko K A, Djordjevic M, Wanner B L and Severinov K 2010 Transcription, processing and function of CRISPR cassettes in *Escherichia coli* *Mol. Microbiol.* **77** 1367