Optimal Gene Circuit Design 2

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Optimal design of gene regulation in varying environments

The lac system

A simple regulation circuit

Glucose starvation

Lactose or IPTG

Optimal design of input functions in varying environments

- Since the *lac* operon is activated by lactose, thus, the input function of Lac production can be modeled as an Hill equation for an activator in the form,

\[ \beta(L) = \frac{\beta_{\text{max}}L^n}{K^n + L^n}. \]

- The parameters of this function have been experimentally determined by fitting the data collected at 1 hour after lactose exposure to this equation:

\[ K = 160 \text{ mM}, \quad n = 4 \]

LacZ activity was determined at t = 1hr
Dynamics of Lac protein production

Thus, when \( L > 160 \, \mu \text{M} \), the input function can be approximated by a logic function

\[
\beta(L) = \beta_{\text{max}} \theta(L > K = 160 \, \mu \text{M}).
\]

The concentration of LacZ protein is then,

\[
Z(t) = \frac{\beta_{\text{max}}}{\alpha} (1 - e^{-\alpha t}).
\]

Assume LacZ is a stable protein, then \( \alpha \) is the dilution rate due to cell growth. The response time of LacZ is,

\[
T_{1/2} = \tau_{\text{cell-cycle}} = \frac{\ln 2}{\alpha}, \text{ then,} \]

\[
\alpha = \ln 2 / \tau_{\text{cell-cycle}}.
\]

Dekel & Alon Nature 2005
Production of Lac proteins under constant conditions

- Under constant lactose levels, we assume that LacZ is always at its steady state level $Z$, thus, protein produced in a cell cycle is also $Z$, i.e. the produced LacZ is diluted by cell volume expansion.

- The concentration of LacZ as well as the amount of protein $Z$ produced in a cell cycle is,

$$ Z = Z(\tau_{cell-cycle}) = \frac{\beta_{\text{max}} \tau_{cell-cycle}}{\ln 2} \left(1 - e^{-\frac{\ln 2}{\tau_{cell-cycle}}}\right), $$

$$ = \frac{\beta_{\text{max}} \tau_{cell-cycle}}{\ln 2} (1 - e^{\ln 2^{-1}}), $$

$$ = \frac{\beta_{\text{max}} \tau_{cell-cycle}}{\ln 2} (1 - \frac{1}{2}), $$

$$ = \frac{\beta_{\text{max}} \tau_{cell-cycle}}{2 \ln 2}. $$
Fitness function of the *lac* operon under constant conditions

- As we have shown earlier, under constant lactose levels, the fitness function of *E. coli* to have the *lac* operon is given by,

\[
G(Z, L) = b(Z, L) - \eta(Z) = \frac{\delta Z L}{K + L} - \frac{\eta_0 Z}{1 - Z / M}.
\]

Where \( G(Z, L) \) is the number of cells that have increased during a period of time \( \tau \); \( Z \) is the concentration of LacZ, as well as amount of LacZ protein that has been produced in \( \tau \); and \( \delta, \eta_0 \) and \( K \) are constants.

- If we consider \( \tau \) as a unit of time, then \( Z \) can be consider as the rate of production of LacZ, i.e., \( \beta = Z / \tau \), then,

\[
G(\beta, L) = \frac{\delta \beta L}{K + L} - \frac{\eta_0 \beta}{1 - \beta / M}.
\]
Optimality in constant environment cannot explain the shape of the input function

- The optimal expression rate of LacZ given at a constant lactose level $L$ is,

$$\beta_{opt} = M (1 - \sqrt{\frac{\eta_0 (K + L)}{\delta L}}).$$

- However, the shape of the input function determined at $t=1$ hour is significantly differently from the predicted optimal expression under different constant levels of lactose $L$, in particular when $L > 1,000 \mu$M, or $L < 400$ mM.

Laboratory evolution of the Lac system in constant conditions

- However, the LacZ activity of *E. coli* cells can evolve to the predicted optimal levels in several hundred generations in constant conditions.

- In other words, if we measure the input function at $t=75$ days, then the input function should give the same expression rate as predicted optimal expression rate.

\[
\frac{\beta_{\text{opt}}(L)}{\beta_{\text{WT}}} = M(1 - \frac{\eta_0(K + L)}{\delta L}) / \beta_{\text{WT}}
\]

\[
\frac{\beta(L)}{\beta_{\text{WT}}} = \frac{L^n}{K^n + L^n}
\]
Is the input function not optimized in variable conditions?

One reason for this discrepancy is that this fitness function tends to overestimate the benefit in a varying condition, in which $Z$ is not uniform; e.g. it is lower at the early times of a pulse of lactose of duration $D$.

When cells are exposed to a pulse of lactose with duration $D$ and level $L$, LacZ is instantly produced at the maximal rate $\beta_{\text{max}}$, which incurs a cost at

$$\eta(\beta_{\text{max}}) = \eta_0 \frac{\beta_{\text{max}}}{1 - \beta_{\text{max}}/M}.$$ 

At any time point in $D$, the benefit is proportional to the level of $Z(t)$,

$$b(\beta, L, t) = \delta \beta_{\text{max}} (1 - e^{-at}) \frac{L}{K + L}.$$
Fitness function under a pulse of lactose

The fitness at any time point in $D$, is,

$$G(\beta_{\text{max}}, L, t) = \delta \beta_{\text{max}} (1 - e^{-at}) \frac{L}{K + L} - \eta_0 \frac{\beta_{\text{max}}}{1 - \beta_{\text{max}} / M}.$$  

Therefore, the fitness function in pulse environments is the integration over $D$,

$$G_{\text{pulse}}(\beta_{\text{max}}, L, D) = \int_0^D G(\beta_{\text{max}}, L, t) dt$$

$$= \int_0^D \delta \beta_{\text{max}} (1 - e^{-at}) \frac{L}{K + L} dt - \eta_0 \frac{D \beta_{\text{max}}}{1 - \beta_{\text{max}} / M}$$

$$= \frac{L \delta \beta_{\text{max}}}{K + L} \int_0^D (1 - e^{-at}) dt - \eta_0 \frac{D \beta_{\text{max}}}{1 - \beta_{\text{max}} / M}$$

$$= \frac{L \delta \beta_{\text{max}}}{K + L} \left(D \frac{e^{-\alpha D}}{\alpha} - 1\right) - \eta_0 \frac{D \beta_{\text{max}}}{1 - \beta_{\text{max}} / M}.$$
Optimal expression of the \textit{lac} operon under a pulse of lactose

Thus the optimal production rate of LacZ in a pulse environment can be found by taking, \(\frac{\partial G_{\text{pulse}}(\beta_{\text{max}}, L)}{\partial \beta_{\text{max}}} = 0\).

From \(G_{\text{pulse}}(\beta_{\text{max}}, L, D) = \frac{L \delta \beta_{\text{max}}}{K + L} \left( D + \frac{e^{-\alpha D} - 1}{\alpha} \right) - \eta_0 \frac{D \beta_{\text{max}}}{1 - \beta_{\text{max}} / M} \), we have,

\[
\frac{L \delta}{K + L} \left( D + \frac{e^{-\alpha D} - 1}{\alpha} \right) - \eta_0 D \frac{1 - \beta_{\text{max}} / M + \beta_{\text{max}} / M}{(1 - \beta_{\text{max}} / M)^2} = 0,
\]

\[
\beta_{\text{max}}(L, D) = \beta_{\text{opt}}(L, D) = M \left( 1 - \frac{\eta_0 (K + L)}{\delta L} \right) \frac{1}{1 + (e^{-\alpha D} - 1) / \alpha D},
\]

where \(A(D) = \frac{1}{1 + (e^{-\alpha D} - 1) / \alpha D} \geq 1\), is called the \textbf{pulse factor}, which is a decreasing function of \(D\). When \(D\) is large, \(A(D) = 1\), which becomes the case of constant environment.
The new fitness function can better explain the experimental data from pulse exposure to lactose

- The optimal LacZ expression levels under pulse lactose exposure are less than those under constant lactose levels, in particular, when lactose levels are lower than 100 and higher than 1000μM.

- This may explain the behavior of the lac operon input function when lactose levels are lower than 100μM or higher than 1000μM.

- However, the predicted optimal lacZ levels are too lower when L is at an intermediate level around 400 μM.

Kalisky T. et at, Phys Biol. 4:229-245, 2007
The optimal expression of Lac proteins is lower in pulse conditions than in constant conditions

- Since the pulse factor $A(D)$ is a decreasing function given a lactose level, the shorter the pulse $D$, the smaller the optimal production rate of LacZ.

- When $D$ is shorter, higher $L$ is needed to obtain the same production rate of LacZ.

- Due to the pulse nature of the presence of lactose in the environment, the wild type $lac$ operon expression is lower than the optimal expression in the constant environment.
Expression of the *lac* operon is not beneficial when lactose pulse is too short

- When D is too short, producing lacZ is not beneficial to the organism, the proteins will not be produced.

- To find such conditions, let’s solve for D in $\beta_{opt}(L,D) = 0$,

$$\beta_{opt}(L,D) = M(1 - \sqrt{\frac{\eta_0(K + L)}{\delta L}A(D)}) = 0,$$

$$\frac{\eta_0(K + L)}{\delta L}A(D) = 1,$$

For a short D, $A(D) \approx 2/(\alpha D)$,

$$\frac{2\eta_0(K + L)}{\alpha \delta LD} = 1,$$

$$D = \frac{2\eta_0(K + L)}{\alpha \delta L}.$$

- For a given level of lactose L, if the pulse is shorter than this D, no lacZ protein will be produced.
Expression of the *lac* open is not beneficial when lactose pulse is too short

- The limit of this $D$ as $L$ approaches infinite gives the critical value of $D$ ($D_c$), any level of lactose pulse $< D_c$ will not induce any production of LacZ.

\[
D_c = \lim_{L \to +\infty} \frac{2\eta_0(K + L)}{\alpha \delta L}
\]

\[
= \lim_{L \to +\infty} \frac{2\eta_0K}{\alpha \delta L} + \lim_{L \to +\infty} \frac{2\eta_0}{\alpha \delta}
\]

\[
= \frac{2\eta_0}{\alpha \delta} = \frac{2\eta_0}{\delta \ln 2} \tau_{\text{cell-cycle}}
\]

\[
= \frac{1}{3} \tau_{\text{cell-cycle}}
\]

\[
= 10 \text{ min.}
\]
Expression of the \textit{lac} open is not beneficial when lactose level is too low

- When L is too low, producing lacZ is not beneficial to the organism either, and thus the proteins will not be produced.

- To find such conditions, let’s solve for L in $\beta_{\text{opt}} (L, D) = 0$, 

\[ M(1 - \sqrt{\frac{\eta_0(K + L)}{\delta L} A(D)}) = 0, \]

\[ \frac{\eta_0(K + L)}{\delta L} A(D) = 1. \]

For a long D, $A(D) \approx 1$, we reduce to the case of a constant L level,

\[ \frac{\eta_0(K + L_c)}{\delta L_c} = 1, \]

\[ L_c = \frac{K}{\delta / \eta - 1} = 55 \mu M. \]

- \textit{E. coli} growing in a media with $L < L_c$ will lose its lac operon.
Optimal expression of Lac operon under a pulse of lactose

Similarly, we can find the relationship between D and L when the optimal expression rate is $\beta_{WT}$.

$$\beta_{opt}(L, D) = M \left(1 - \sqrt{\frac{\eta_0(K + L)}{\delta L} A(D)}\right) = \beta_{TW},$$

$$\frac{\eta_0(K + L)}{\delta L} A(D) = (1 - \frac{\beta_{TW}}{M})^2,$$

For a short D, $A(D) \approx 2/(\alpha D)$,

$$\frac{2\eta_0(K + L)}{\alpha \delta L D} = (1 - \frac{\beta_{TW}}{M})^2,$$

$$D = \frac{2\eta_0(K + L)}{\alpha \delta L (1 - \frac{\beta_{TW}}{M})^2},$$

$$= \frac{2\eta_0(K + L)}{\delta L (1 - \frac{\beta_{TW}}{M})^2 \ln 2} \tau_{cell-cycle}.$$
Optimal expression of Lac operon under a pulse of lactose

The minimal duration $D_c$ to have $\beta_{\text{opt}} = \beta_{\text{WT}}$ can be found by,

$$D_c = \lim_{{L \to +\infty}} \frac{2\eta_0(K + L)}{\delta L(1 - \frac{\beta_{\text{TW}}}{M})^2 \ln 2} \tau_{\text{cell-cycle}},$$

$$= \frac{2\eta_0}{\delta(1 - \frac{\beta_{\text{TW}}}{M})^2 \ln 2} \tau_{\text{cell-cycle}},$$

$$= 3\tau_{\text{cell-cycle}}.$$

Thus, when the duration of a pulse is less than 3 cell generation, it can never have $\beta_{\text{WT}}$ as its optimal expression rate.
Expression of lac open can never be optimal when lactose level is too low

- When L is below a critical Lc level, the production of LacZ cannot be optimal no matter how long D is. To find such conditions, let’s solve for L in $\beta_{opt}(L,D) = \beta_{wt}$,

$$M(1 - \sqrt{\eta_0(K + L)/\delta L} A(D)) = \beta_{wt},$$

$$\eta_0(K + L)/\delta L A(D) = (1 - \beta_{wt}/M)^2,$$

For a long D, $A(D) \approx 1$,

$$\eta_0(K + L_c)/\delta L_c = (1 - \beta_{wt}/M)^2,$$

$$\eta_0 M^2(K + L_c) = \delta L_c(M - \beta_{wt})^2,$$

$$L_c = \frac{\eta_0 M^2 K}{\delta(M - \beta_{wt})^2 - \eta_0 M^2},$$

$$D_c = \frac{2\eta_0}{\tau \delta \ln 2} = \frac{1}{3}$$

$$L_c = 570 \mu M.$$

- *E. coli* grown in a media with $L<L_c$ will evolve to low its LacZ expression.
Optimal expression of Lac operon under a pulse of lactose

- Therefore, bacteria exposed to pulses shorter than one third of cell generation gain no benefit from the lac operon.
- This conclusion is consistent with the view that genetic circuits in rapidly changing environments are effective only if their response time is equal to or faster than the rate of the change of the environmental signals.
- Too prolonged low level of lactose is not beneficial to bacteria, which may leads to the loss of the lac operon.
- Under pulse conditions the optimal expression of the lac operon is lower than in constant conditions.
- This may explain the behavior of the lac operon input function when lactose levels are lower than 200 or higher than 1000μM.