I) Reaction

$$2\,LacI + IPTG \underset{k_3}{\overset{k_2}{\rightleftharpoons}} IPTG\text{-}LacI_2 \qquad K_\alpha = k_2/k_3$$

$$2\,LacI + P \underset{k_5}{\overset{k_4}{\rightleftharpoons}} P\text{-}LacI_2 \qquad K_P = k_4/k_5$$

$$IPTG + P\text{-}LacI_2 \underset{k_7}{\overset{k_6}{\rightleftharpoons}} P + IPTG\text{-}LacI_2 \qquad K_\gamma = k_6/k_7$$

Assumptions

* no intermediary P_IPTG-LacI₂ complex

stochiometric effects || Binding of LacI to IPTG can be more complex
we assume 2 LacI for 1 IPTG ...

II Before IPTG is introduced ...

$$\begin{cases} \dfrac{dGFP}{dt} = k_8[P] - d_{GFP}[GFP] \\ \dfrac{d[LacI]}{dt} = k_1 - d_1[LacI] + k_5[P\text{-}LacI_2] - k_4[P][LacI]^2 \\ \dfrac{dP}{dt} = -k_4[P][LacI]^2 + k_5[P\text{-}LacI_2] \\ \qquad = -\dfrac{d[P\text{-}LacI_2]}{dt} \end{cases}$$

IPTG introduced @ steady state of previous system

$$[LacI] = k_1/d_1$$

$$[P\text{-}LacI_2] = K_P[P][LacI]^2$$

conservation of promoters $P_0 = P + [P\text{-}LacI_2]$

$$[P\,LacI_2] = \frac{K_P[LacI]^2}{1 + K_P[LacI]^2}$$

$$P = \frac{P_0}{1 + K_P[LacI]_{ss}^2}$$

$$[GFP]_{ss} = \frac{k_8 P_0}{(1 + K_P[LacI]_{ss}^2) \times d_{GFP}}$$ // basal concentration

1

III IPTG is now introduced

steady state expression of LacI, unbinding of bound LacI, Binding of free LacI

$$\frac{d[lacI]}{dt} = k_1 - d_1[lacI] + k_5[P-lacI_2] - k_4[P][lacI]^2$$
$$- k_2[IPTG][lacI]^2 + k_3[IPTG-lacI_2]$$

Binding of free LacI, Unbinding of bound LacI.

$$\frac{d\,IPTG}{dt} = -k_2[IPTG][lacI]^2 + k_3[IPTG-lacI_2]$$

Binding of free IPTG, unbinding of bound IPTG

$$- k_6[IPTG][P-lacI_2] + k_7[P][IPTG-lacI_2]$$

Binding of free IPTG, Unbinding of bound IPTG.

LacI-IPTG complex

$$\frac{d[IPTG-lacI_2]}{dt} = k_2[lacI]^2[IPTG] - k_3[IPTG-lacI_2]$$

Complex formation, complex dissociation.

$$+ k_6[IPTG][P-lacI_2] - k_7[P][IPTG-lacI_2]$$

complex formation, complex deformation.

P = ~~total~~ free promoter

$$\frac{dP}{dt} = -k_4[P][lacI]^2 + k_5[P-lacI_2] - k_7[P][IPTG-lacI_2]$$

Complex ~~dissociation~~ formation, complex dissociation.

$$+ k_6[IPTG][P-lacI_2]$$

complex formation, complex dissociation

P-lacI₂ = Promoter-LacI complex.

Complex formation, complex dissociation

$$\frac{d[P-lacI_2]}{dt} = +k_4[P][lacI]^2 - k_5[P-lacI_2]$$
$$- k_6[IPTG][P-lacI_2] + k_7[P][IPTG-lacI_2]$$

complex dissociation, complex formation.

$\boxed{\text{@ steady state all } \frac{d}{dt} = 0}$

$$\Rightarrow \begin{cases} (lacI)_{ss} = k_1/d_1 \\ (IPTG-lacI_2) = K_\alpha [lacI]^2_{ss}[IPTG] \\ [P-lacI_2] = K_\beta [P][lacI]^2_{ss} \\ [P][IPTG\,lacI_2] = K_\gamma [IPTG][P-lacI_2] \quad (*) \end{cases}$$

$$\begin{cases} P_0 = P + [P-lacI_2] \\ I_0 = [IPTG] + [IPTG-lacI_2] \end{cases}$$

⇒ Problem!!

Last equation (*) not compatible

⇒ We need complex P-IPTG-lacI₂ !!

IV Looking for the Steady States : $\frac{d}{dt}$ of everything $\Rightarrow 0$.

Temporary Notations:

$$A = K_2[IPTG][LacI]^2 - K_3[IPTG\text{-}LacI_2]$$

$$B = K_4[P][LacI]^2 - K_5[P\text{-}LacI_2]$$

$$C = K_6[IPTG][P\text{-}LacI_2] - K_7[P][IPTG\text{-}LacI_2]$$

* @ steady states we have

$$\begin{cases} A + C = 0 & \text{from } d[IPTG]/dt = 0 \\ B - C = 0 & \text{from } dP/dt = 0 \\ K_1 - d_1[LacI] - B - A = 0 & \text{from } d[LacI]/dt = 0 \end{cases}$$

$\Rightarrow A + C + B - C = 0 + 0 \Rightarrow A + B = 0$.

$\leadsto [LacI] = K_1/d_1$

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* Conservation Equations

$$\begin{cases} P_0 = [P] + [P\text{-}LacI_2] & \text{Conservation of promoter} \\ I_0 = \underset{\overset{\shortparallel}{I}}{[IPTG]} + [IPTG\text{-}LacI_2] & \text{Conservation of IPTG.} \end{cases}$$

$\swarrow$ from conservation equation

$\leftarrow$ from conservation equation.

$$A + B = K_2[LacI]^2[IPTG] - K_3(I_0 - I) + K_4 P[LacI]^2 - K_5(P_0 - P) = 0$$

Rearranging the above:

$$(K_2[LacI]^2_{ss} + K_3)I + (K_4[LacI]^2 + K_5)P = K_3 I_0 + K_5 P_0$$

Notation ; $\alpha = [LacI]^2_{ss}$.

** Notation $a = [LacI]^2_{ss}$

Rewriting

$$(1) \quad (aK_2 + K_3)I + (aK_4 + K_5)P = K_3 I_0 + K_5 P_0$$

In a similar manner:

$$A + C = K_2 a I - K_3(I_0 - I) + K_6 I(P_0 - P) - K_7 P(I_0 - I) = 0$$

$$\Rightarrow (2) \quad (aK_2 + K_3 + K_6 P_0)\boxed{I} - K_7 I_0 P + (K_7 - K_6) I.P = K_3 I_0$$

how has he substituted $(\alpha k_2 + k_3)I$ for I ?

$$\boxed{(aK_2 + K_3)I} = K_3 I_0 + K_5 P_0 - (aK_4 + K_5)P$$

Solve simultaneously.

and put the $(\alpha k_2 + k_3)$ here.

Aha! Divide (1) by $(\alpha k_2 + k_3)$ and mult thro

** Substitute I into (2) $\Longrightarrow$

$$(aK_2 + K_3 + K_6 P_0)(K_3 I_0 + K_5 P_0 - (aK_4 + K_5)P) - K_7 I_0 (aK_2 + K_3)P$$
$$(3) \quad + (K_7 - K_6)P(K_3 I_0 + K_5 P_0 - (aK_4 + K_5)P) = K_3 I_0 (aK_2 + K_3)$$

and Therefore Rewriting:

$$\underbrace{P^2 (K_6-K_7)(aK_4+K_5)}_{\text{terms in } P^2} - \underbrace{P\Big((aK_4+K_5)(aK_2+K_3+K_6P_0) + K_7I_0(aK_2+K_3) + (K_6-K_7)(K_3I_0+K_5P_0)\Big)}_{\text{terms in } P'}$$

terms not in P.

$$+ K_7 I_0 (aK_2+K_3) = 0$$

which can be rewritten as How.?.

$$P^2(K_6-K_7)(aK_4+K_5) - P\Big((aK_4+K_5)(aK_2+K_3+K_6P_0) + K_5P_0(K_6-K_7)\Big)$$
$$+K_5 P_0 (aK_2+K_3+K_6P_0)$$
$$+ I_0 (P_\infty - P)\times(K_3K_6+aK_2K_7) = 0 \quad P_\infty = \frac{P_0 \text{ Bound}}{1+aK_\alpha/K_\gamma}$$

What does this represent? P at $I=\infty$.

as a proportion of all.

P_∞ = ~~[illegible]~~ $P_\infty \sim P_0$

Initial condition $P = \frac{P_0}{1+aK_\beta}$ ← & initial promotes

1+ Bound promotes

$aP_\infty \sim P_0$ & [P] when $I_0 = \infty$

This suggests that we solve with $X = P/P_0$

X = proportion of promoters that will express GFP

The equation can be rewritten as

$$X^2 (K_6-K_7)(aK_4+K_5) - X\left(K_5(K_6-K_7) + (aK_4+K_5)\frac{(aK_2+K_3+K_6)}{P_0}\right)$$
$$+ K_5\left(\frac{aK_2+K_3}{P_0} + K_6\right) + \underbrace{I_0 (X_\infty - X)\frac{K_3K_6+aK_2K_7}{P_0}}_{R(X)\times(K_6-K_7)(aK_4+K_5)} = 0$$

How have we gotten this P^2 to $= P^2/P_0^2$

$Q(X)\times(K_6-K_7)(aK_4+K_5)$

notation $b = \frac{aK_2+K_3}{P_0} + K_6$

$$Q(X) = \cancel{(K_6-K_7)(aK_4+K_5)}\left\{X^2 - X\left\{\frac{K_5}{aK_4+K_5} + \frac{b}{K_6-K_7}\right\} + \frac{K_5}{aK_4+K_5}\times\frac{b}{K_6-K_7}\right\}$$

roots of Q: $\underbrace{\frac{K_5}{aK_4+K_5} = \frac{1}{aK_\beta+1}}_{X_1}$; $\frac{b}{K_6-K_7}$

corresponds to $I_0=1$?

corresponds to $I_0 = 1$

4

4b ii)

$$C = \frac{k_3 k_6 + a k_2 k_7}{P_0 (k_6 - k_7)(a k_4 + k_5)} = \frac{k_3 (k_\gamma + a k_\alpha)}{P_0 (k_\gamma - 1)(a k_4 + k_5)}$$

$$R(X) = C(X_\alpha - X)$$

V And Now for the solutions

* The easy part

$Q(X)$ has at least one positive solution

$X_1 = \frac{1}{\alpha K_p + 1}$ (≡ the steady state if $I_0 = 0$)

$X_2 = \frac{b}{K_6 - K_7}$ is also acceptable if $K_7 \lessgtr 1$

$R(X)$ has only one root X_∞

as $I_0 \to \infty$ ~~$R(X) = I_0 R(X)$ degenerates~~ ~~the~~ $I_0 R(X)$ in $Q(X) + I_0 R(X)$ increases the weight of

In particular the roots (complex or not) converge to X_∞

** First Case $K_6 < K_7$ $(c < 0)$

$Q(X)$ has only one root that is positive

For $I_0 > 0$, we seek the roots of

$$(X - X_1)(X - X_2) + c I_0 (X_\infty - X) = 0$$

To make the calculation easier we let

$Y = X - X_1$ $\quad Y_2 = X_2 - X_1 \ (<0)$ $\quad Y_\infty = X_\infty - X_1$

$$Y(Y - Y_2) + c I_0 (Y_\infty - Y) = 0$$

$$Y^2 - Y(Y_2 + c I_0) + c I_0 Y_\infty = 0$$

$$Y = \frac{Y_2 + c I_0 \pm \sqrt{(Y_2 + c I_0)^2 - 4 c I_0 Y_\infty}}{2}$$

$+$ for I_0 close to zero

~~###~~ $K_6 < K_7$ and $X_\alpha > X_1$ The Nice Case

$Y_\alpha > 0$ → discriminant equation always > 0

$$Y = \frac{Y_2 + cI_0 + \sqrt{(Y_2 + cI_0)^2 - 4cI_0 Y_\alpha}}{2}$$

Evolution of the solution

$$2\frac{dY}{dI_0} = c + \frac{1}{2\sqrt{\ }}\left(-4cY_\alpha + 2c(Y_2 + cI_0)\right)$$

$$= \frac{c}{\sqrt{\ }}\left(\sqrt{\ } - 2Y_\alpha + Y_2 + cI_0\right)$$

@ $I_0 = 0$ $\frac{dY}{dI} = \frac{-2cY_\alpha}{|Y_2|} > 0$

$$\text{sgn}\frac{dY}{dI_0} = -\text{sgn}\left(\sqrt{\ } \underbrace{- 2Y_\alpha + Y_2 + cI_0}_{<0}\right)$$

$$= -\text{sgn}\left((Y_2 + cI_0)^2 - 4cI_0 Y_\alpha - (Y_2 + cI_0 - 2Y_\alpha)^2\right)$$

$$= -\text{sgn}\left(-4c\cancel{I_0}Y_\alpha - 4Y_\alpha^2 + 4Y_\alpha(Y_2 + c\cancel{I_0})\right) > 0$$

⇐ we can have slow start, or fast start, to the curve → Hill coeff can be anything

Complement: what does $X_\alpha > X_1$ mean?

it means $K_\beta > K_\alpha / K_\gamma$

K_β: dissociation rate of the 'repression' loop

$LacI \rightarrow LacI_2 - P$ (direct binding)

When IPTG is introduced, another way to repress is enabled: the 'backdoor way'

$LacI \rightarrow IPTG\text{-}LacI_2 \rightarrow LacI_2 - P$

The dissociation constant of the other route is $K\alpha / K\gamma$

If this route is more attractive than the direct route ($K\alpha/K\gamma > K_D$), we repress even more $\Rightarrow$ $[GFP]_\alpha < [GFP]_{\alpha=0}$

If on the contrary, the backdoor route is less attractive adding this route will hamper repression $\Rightarrow$ $[GFP]_{\alpha\to\alpha} > -$

$\cancel{*}\cancel{*}\cancel{*}$ $K_6 < K_7$ & $X_\alpha < X_1$

The discriminant of the equation is

$$\Delta = (Y_2 + cJ_0)^2 - 4cJ_0 Y_\alpha$$

that is

$$\Delta = X^2 + X(2Y_2 - 4Y_\alpha) + Y_2^2 \qquad X = cJ_0$$

we have a solution iff $\Delta \geq 0$

$\Delta(X)$ poly in X

For $X > 0$, $\Delta(X) \geq 0$ iff $2Y_2 - 4Y_\alpha \leq 0$ ie $\boxed{Y_2 \leq 2Y_\alpha}$

(both possible roots ≤ 0)

or $\Delta_2 = (2Y_2 - 4Y_\alpha)^2 - 4Y_2^2 < 0$

(no real solutions)

ie $-16Y_2Y_\alpha + 16Y_\alpha^2 < 0$

$Y_\alpha(Y_\alpha - Y_2) < 0$

ie $Y_\alpha \geq Y_2$

$\| \Delta(X) \geq 0$ iff $Y_2 \leq 2Y_\alpha$

Then we have $Y(J_0)$ $\forall$ fn of J_0

if $0 > Y_2 > Y_\alpha$, then for $J_0 > J_{0,\max}$,

there are no <u>real</u> steady points !!

But it is impossible $X_2 < 0$; $X_\alpha > 0$

$\Rightarrow Y_2 < Y_\alpha$

Thanks

8 Second case $K_6 > K_7$ $C > C$

$Q(X)$ has 2 real, positive solutions,

* First important result: for $K_6 > K_7$ $X_2 > X_1$ and $X_2 > X_\infty$

proof $b = K_6 + \frac{\alpha K_6 K_3}{\beta_0} > K_6$

$$\Rightarrow \underbrace{\frac{b}{K_6 - K_7}}_{X_2} > 1$$

and $X_1 = \frac{1}{1+\alpha K_\beta} < 1$ (same for X_∞)

Good news: this means fewer cases to study,

** First Possibility $X_\infty < X_1$ ie $Y_\infty < 0$

As before we now seek the roots of

$$Y(Y - Y_2) + cI_0(Y_\infty - Y) = 0$$

that is $Y^2 - Y(Y_2 + cI_0 Y_\infty) + \underbrace{cI_0 Y_\infty}_{<0} = 0$

2 roots of opposite signs

The root we are interested in is

$$Y = \frac{Y_2 + cI_0 Y_\infty - \sqrt{(Y_2 + cI_0 Y_\infty)^2 - 4cI_0 Y_\infty}}{2} < 0$$

$Y(I_0)$ decreasing function $\begin{cases} Y(0) = Y_1 = 0 \\ Y(+\infty) = Y_\infty \end{cases}$

** Other Possibility $Y_\infty > 0$

The discriminant of the equation is

$(Y_2 + cI_0 Y_\infty)^2 - 4cI_0 Y_\infty > (Y_\infty + cI_0 Y_\infty)^2 - 4cI_0 Y_\infty > 0$

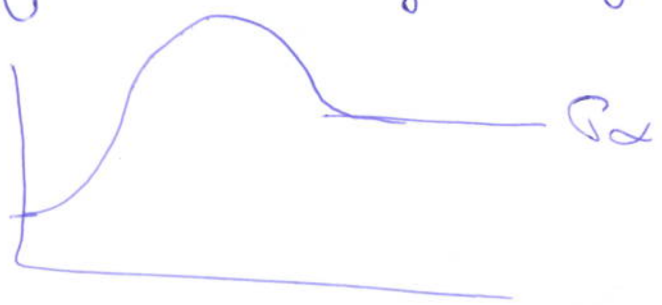
no problem

$$Y(I_0) = \frac{Y_2 + cI_0 Y_\infty - \sqrt{(Y_2 + cI_0 Y_\infty)^2 - 4cI_0 Y_\infty}}{2}$$

growing function of I_0

VI Final Thoughts...

- Simulations confirm the results obtained in the analysis
* Influence of degradation terms non negligible.
  - if d_1 large enough, $P(t)$ is not monotonous



  - Therefore if d_{GFP} large enough, GFP also exhibits a bump!!
  - degradation terms ~~not~~ in practice will likely be small $\Rightarrow$ my prediction is no bump!

* $K_{back} = K_\alpha + K_\gamma$ and K_β determine the dynamics of the construct
  The larger K_β / K_{back} is, the larger the gain in fluorescence is
* $[GFP]_{ss}(I_0)$ can be approximated by a Hill function in practice (albeit an offset one!)
* Ideal Construct

$$\begin{cases} K_\beta (k_1/d_1)^2 \text{ large} \\ (K_\alpha + K_\gamma) \times (k_1/d_1)^2 \text{ low} \end{cases} \rightarrow \text{we want } \begin{array}{l} K_\beta \text{ large} \\ K_\alpha + K_\gamma \text{ low} \\ n \text{ large.} \end{array}$$

$\Rightarrow$ Constitutive promoter: as strong as possible and of course K_γ as large as possible