

- 1.) Assumptions:
- all experiments conducted in exponentially growing pop. of *E. coli* w/ doubling time  $T_d \approx 40$  min.
  - $OD_{600} = 0.1$  equivalent to  $1 \times 10^8$  cells/ml
  - inducer transport ignored for promoter func. in terms of extracellular inducer
  - lacZ* gene present @ 2 copies/cell
  - lacZ* mRNA half-life is 5 minutes
  - transcript length = 1000 nt

a)  $\langle n \rangle \Rightarrow B = \langle m_c \rangle N_c V$  ← sample volume (mL)

$\begin{matrix} \uparrow \\ \text{avg mass} \\ \text{cell} \end{matrix}$        $\begin{matrix} \uparrow \\ \text{cell count} \\ (\text{cells}/\text{cell}) \end{matrix}$   
 $(\text{gDW}/\text{cell})$        $(\text{cells}/\text{mL})$

$$V = 1 \text{ mL}$$

$$\hat{N}_c = 1 \times 10^8 \text{ cells/mL}$$

$$\langle m_c \rangle = 2.8 \times 10^{-13} \text{ g/cell} \quad \text{BIND: } 103904$$

↓ convert to gDW/cell...

~0.30 dry cell

$$\frac{2.8 \times 10^{-13} \text{ g/cell}}{30} \times 100 = 9.33 \times 10^{-13} \text{ gDW/cell}$$

$$B = (9.33 \times 10^{-13})(1 \times 10^8)(1) = 9.33 \times 10^{-5} \text{ gDW}$$

Table 1 conversion of  $\langle n \rangle$  to  $\langle n \rangle / B$

IPTG (mM)	$\langle n \rangle / B$ ( $\frac{\text{mRNA}}{\text{(cell)} \cdot \text{gDW}}$ )
0	$2.036 \times 10^5$
$5 \times 10^{-4}$	$2.25 \times 10^5$
0.005	$4.393 \times 10^5$
0.012	$7.179 \times 10^5$
0.053	$9.214 \times 10^5$
0.216	$9.964 \times 10^5$
1	$9.964 \times 10^5$

$$m_i = r_{x,i} \bar{u}_i - (\nu + \theta_{m,i}) m_i$$

↑  
promoter activity func. [0, 1]

↓  
specific rate  
of transcription i

b constant

$\therefore \dot{B} = 0$

$K = \frac{\delta(\text{output var})}{\delta(\text{input var})} = \frac{\Delta \text{output var}}{\Delta \text{input var}}$

$\therefore \nu = \beta \beta'$

$\frac{dm_i}{dt} = r_{x,i} \bar{u}_i - \theta_{m,i} m_i$

$$\frac{dm(s)}{ds} + m(s) = m_{in}(s) - m_{out}(s)$$

$$(s + \theta_{m,i}) M(s) = m_{in}(s)$$

gain

$$K = \frac{m(s)}{\bar{u}_i(s)} = \frac{r_{x,i}}{s + \theta_{m,i}} = \frac{\frac{r_{x,i}}{\theta_{m,i}}}{s + 1}$$

Pseudo steady state  $\Rightarrow \frac{dm}{dt}$  fast  $\therefore \frac{dm}{dt} = 0$

$$0 = r_{x,i} \bar{u}_i - \theta_{m,i} m^*$$

gene expression ( $V_{max}$ )

$$\theta_{m,i} m^* = r_{x,i} \bar{u}_i$$

$$m^* = \frac{r_{x,i} \bar{u}_i}{\theta_{m,i}}$$

$$\Rightarrow r_{x,i} = K_{E,i} R_{x,T} \left( \frac{G_i}{I_{x,i} K_{x,i} + (I_{x,i} + 1) G_i} \right)$$

$$\Rightarrow \bar{u}_i = W_1 + W_2 f_i$$

$$\frac{1}{1 + W_1 + W_2 f_i}$$

where

$$f_i = \frac{I^n}{P^{n+1} + I^n}$$

$W$  are micro state weights

$$m^* = K_x(G, f) \bar{u}(I, \gamma)$$

$$* \tau_d = 40 \text{ min} \Rightarrow 2400 \text{ s}$$

I don't know where  
to add  
cell growth

$$K_x = K_{E,i}^x R_{x,T} \left( \frac{G_i}{T_{x,i} K_{x,i} + (T_{x,i} + 1) G_i} \right)$$

$$\theta_{m,i} = \frac{1}{5 \text{ min}} \downarrow \\ \frac{1}{300 \text{ s}}$$

c) Assumption:  $P_{lac} \Rightarrow$  positively inducible promoter that responds to IPTG  $\rightarrow \text{nmol}$

$$\rightarrow G_i = \text{lacZ gene conc.} = 2.10^{12} \text{ S} \left( \frac{1}{9.33 \times 10^{-13}} \right) \left( \frac{1}{6.02 \times 10^{23}} \right) \left( 1 \times 10^9 \right) = 0.0356 \frac{\text{nmol}}{\text{gdw}}$$

$$\rightarrow R_{x,T} = 0.0356 \frac{\text{nmol}}{\text{gdw}}$$

$$\rightarrow K_{x,i} = 0.0136 \frac{\text{nmol}}{\text{gdw}} \left( \frac{\text{IPTG}}{1000 \text{ nmol}} \right) \left( \frac{\text{mL}}{0.30 \text{ gdw}} \right) \left( \frac{1000 \text{ nmol}}{1 \text{ mL}} \right) = 0.453 \frac{\text{nmol}}{\text{gdw}}$$

$$\rightarrow K_E^x = \text{ex/l} = \frac{25}{1000} = 0.025 \frac{1}{\text{l}}$$

$$K_I = K_2 = 0.0455 \frac{1}{\text{l}}$$

$$\rightarrow T_{x,i} = \frac{K_E^x}{K_I} = \frac{0.025}{0.0455} = 0.549$$

tot 50%

$$K_x = (300)(0.025)(0.00356) \left( \frac{0.00356}{0.549(0.0453) + (0.549+1)(0.00356)} \right) = 0.00313 \text{ IPTG (mM)}$$

$\bar{u}$  function...

$$f_I = \frac{I^n}{K^n + I^n}$$

let  $n = 5$  ( $I$  really small)

IPTG	RNAPII	$P_{lac}$	$K^n + I^n$	
weights:	0	0	$\uparrow$	
each	1	0	49.6 $\mu\text{M}$ BIND	: 101976
	0	1	$\downarrow$	
W.f_I	1	1	0.0496 mM	

let  $= 5$  (dark, non)

$$\bar{u} = \frac{W_f f_I}{3 + W_f f_I}$$

IPTG (mM)	$K_x$	$\bar{u}$	$m^*$	$\langle n \rangle / B$
0	0.00313	0	0	$2.036 \times 10^5$
1.67		0.624	0.0195	$2.25 \times 10^5$
16.67		0.625	0.0196	$4.393 \times 10^5$
40				$7.179 \times 10^5$
176.67				$9.214 \times 10^5$
720				$9.964 \times 10^5$
3333				$9.964 \times 10^5$

d) Done w/ MATLAB + in plot doc

The model fit has the right idea  
(S-shaped); the parameter  $k$

assume would control  $B$  + shape is the  
 $\bar{u}$  function with the Hill coefficient  
+ fraction involved.

S  
①  
②

$$2.) \text{a) } \frac{d\tilde{X}}{dt} = \tilde{\alpha}_x + \tilde{\beta}_x S - \tilde{\delta}_x \tilde{X}$$

$$\quad \quad \quad 1 + S + (\tilde{Z}/\tilde{z}_x)^{n_{zx}}$$

$$\frac{d\tilde{Z}_1}{dt} = \frac{\tilde{\alpha}_z}{1 + (\tilde{X}/\tilde{x}_z)^{n_{xz}}} - \tilde{\delta}_z \tilde{Z}$$

$$\text{b) } \tilde{\delta}_z = \frac{\tilde{\delta}_z}{\tilde{\alpha}_x} \quad t = \tilde{t} \tilde{\delta}_x$$

$$\tilde{\alpha}_x = \frac{\tilde{\alpha}_x}{\tilde{\alpha}_z} \quad \tilde{\beta}_x = \frac{\tilde{\beta}_x}{\tilde{\alpha}_z} \quad \tilde{z}_x = \frac{\tilde{z}_x \tilde{\delta}_x}{\tilde{\alpha}_z} \quad \tilde{x}_z = \frac{\tilde{x}_z \tilde{\delta}_x}{\tilde{\alpha}_z}$$

$$\tilde{X} = \frac{\tilde{X} \tilde{\delta}_x}{\tilde{\alpha}_z} \quad \tilde{Z} = \frac{\tilde{Z} \tilde{\delta}_x}{\tilde{\alpha}_z}$$

$$\frac{\tilde{\delta}_x}{\tilde{\alpha}_z} \left( \frac{d\tilde{X}}{dt} \right) = \left( \frac{\tilde{\alpha}_x + \tilde{\beta}_x S}{1 + S + (\tilde{Z}/\tilde{z}_x)^{n_{zx}}} \right) \frac{\tilde{\delta}_x}{\tilde{\alpha}_z} \left( \frac{\tilde{\delta}_x \tilde{X}}{\tilde{\alpha}_z} \right) \frac{\tilde{\delta}_x}{\tilde{\alpha}_z}$$

$$\frac{d\tilde{X}}{dt} = \frac{(\tilde{\alpha}_x + \tilde{\beta}_x S) \tilde{\delta}_x}{1 + S + (\tilde{Z}/\tilde{z}_x)^{n_{zx}}} - \tilde{\delta}_x \tilde{X}$$

$$\frac{d\tilde{X}}{dt} = \frac{(\tilde{\alpha}_x + \tilde{\beta}_x S) \tilde{\delta}_x}{1 + S + (\tilde{Z}/\tilde{z}_x)^{n_{zx}}} - \tilde{\delta}_x \tilde{X}$$

$$\boxed{\frac{d\tilde{X}}{dt} = \frac{\tilde{\alpha}_x + \tilde{\beta}_x S}{1 + S + (\tilde{Z}/\tilde{z}_x)^{n_{zx}}} - \tilde{X}}$$

$\frac{\tilde{\delta}_z \tilde{Z}}{\tilde{\alpha}_x \tilde{X}}$   
 $\frac{\tilde{\alpha}_z \tilde{Z}}{\tilde{\delta}_x \tilde{X}}$

$$\frac{\tilde{\delta}_x}{\tilde{z}_2} \left( \frac{d\tilde{z}_1}{dt} \right) = \left( \frac{\tilde{\alpha}_x}{1 + (\tilde{x}/\tilde{x}_2)^{n_{xz}}} - \tilde{\delta}_2 \tilde{z}_1 \right) \frac{\tilde{\delta}_x}{\tilde{z}_2}$$

$$\frac{d\tilde{z}_1}{dt} = \left( \frac{\tilde{\delta}_x}{1 + (\tilde{x}/\tilde{x}_2)^{n_{xz}}} - \tilde{\delta}_2 \tilde{z}_1 \right) \frac{1}{\tilde{\delta}_x}$$

$$\frac{d\tilde{z}_1}{dt} = \frac{1}{1 + (\tilde{x}/\tilde{x}_2)^{n_{xz}}} - \tilde{\delta}_2 \tilde{z}_1$$

c) DONE IN MATLAB Yes, the solid black lines are qualitatively reproducible.  
+ in plot doc

d) DONE IN MATLAB

+ in plot doc

e) let  $S = 0.4$

$$X_{ss} = 0.002$$

$$Y_{ss} = 0.283$$

$$Z_{ss} = 0.00135$$

below Hopf

DONE IN MATLAB + in plot doc

$\hookrightarrow$  oscillations concerning Hopf bifurcation are incoherent  
below

let  $S = 7744$

above saddle

$$X_{ss} = 5.852$$

$$Y_{ss} = 0.000786$$

$$Z_{ss} = 0.000375$$

DONE IN MATLAB + in plot doc

$\hookrightarrow$  oscillations concerning saddle node bifurcation are coherent  
above diagram

When regarding the oscillatory spiral center, oscillations originating from Hopf bifurcation start their transient close to the unstable spiral center and small initial variations  $\Rightarrow$

in later stages

are amplified, so the oscillations lack coherence. On the other hand, oscillations originating from saddle node bifurcation start far from the unstable spiral center, and so when small initial differences are present, they will not be amplified in later stages to make the oscillations indistinguishable.

\* Note: The diagram is essentially an oscillator phase portrait in the gene expression plane of steady states for  $S_5$ .

My results support this as well since the steady-states starting below Hopf bifurcation point did not have easily identifiable oscillations and immediately looking like a block of oscillations. However, the steady-states, starting above saddle node bifurcation had identifiable oscillators at the beginning of the  $S$  value change. (Granted, I used a long time span so later the oscillations aren't as identifiable, but that is due to the fact that the gene expression reached the expected oscillatory outcome when  $S=100$ , whether the origination was below a Hopf or above a saddle node.)

f) It is possible since the change of time and the  $S$  value step from 105 to 108 are small compared to other thus viewing plots. Plus when analyzing the different results of behavior in Figure S.2 for different score results during the optimization process, the scores closest to the parameters used more damped and distinguishable oscillation than higher parameters. Lastly, since Figure 3.5 shows that Z gene expression was in stable steady state beforehand and went through the saddle-node bifurcation ( $S_5$ ) the oscillations are likely to be distinguishable.