Advanced features of Boolean models

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Overview

In this section, we'll see how to add the following features to Boolean models:

- medium levels of protein concentation;
- bistability under medium concentrations;
- time-delays due to cellular processes.
- dilution of protein concentation due to cellular growth;
- degradation (or decay) of protein concentration;

We've already seen how ODE models can incorporate these features.

We will start with a published Boolean model of the *lac* operon that exhibits bistability.

Then, we will build Boolean models derived from the 3-variable and 5-variable ODE models of the *lac* operon, with these advanced features.

Medium concentrations and Bistability

In order for a *lac* operon model to exhibit bistability, it must be able to incorporate medium levels of concentration.

One way to do this is to work over $\mathbb{F}_3 = \{0, 1, 2\}$. However, this increases the state space size from 2^n to 3^n.

Some models have Boolean and ternary variables, but this lacks a nice algebraic framework.

GINsim is able to handle such logical models.

An alternative is to introduce a new variable L_m meaning "at least medium levels" of lactose. Clearly, $L = 1$ implies $L_m = 1$.

In other words:

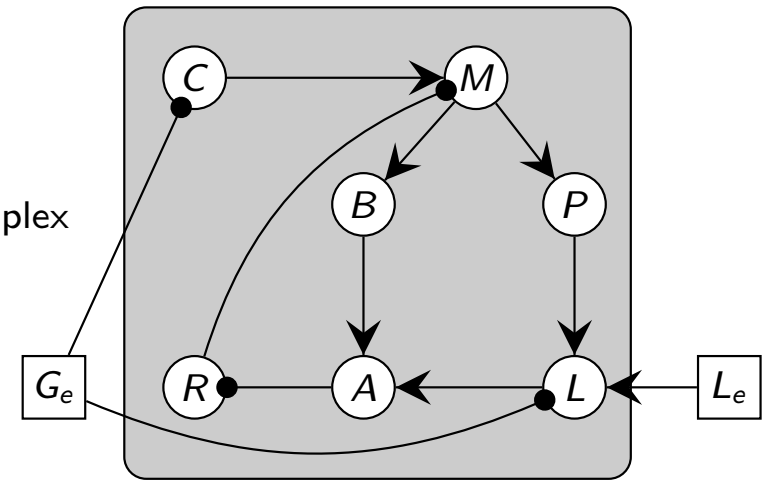
- High lactose: $L = 1$, $L_m = 1$.
- Medium lactose: $L = 0$, $L_m = 1$.
- Low lactose levels: $L = 0$, $L_m = 0$.

We can ignore any state for which $L = 1$, $L_m = 0$.

A Boolean network model of the *lac* operon

Consider the following Boolean network model, which was published in Veliz-Cuba / Stigler (2011).

$M =$ mRNA
$B =$ β-galactosidase
$P =$ *lac* permease
$C =$ cAMP-CAP complex
$R =$ repressor protein
$L =$ lactose
$A =$ allolactose
$G =$ glucose



$$\begin{cases} f_M = \overline{R} \wedge \overline{R_m} \wedge C \\ f_B = M \\ f_P = M \\ f_C = \overline{G_e} \\ f_R = \overline{A} \wedge \overline{A_m} \\ f_{R_m} = (\overline{A} \wedge \overline{A_m}) \vee R \\ f_A = L \wedge B \\ f_{A_m} = L \vee L_m \\ f_L = \overline{G_e} \wedge P \wedge L_e \\ f_{L_m} = \overline{G_e} \wedge ((L_{em} \wedge P) \vee L_e) \end{cases}$$

Comments

- The shaded region represents the cell.
- Circles denote variables, and squares denote parameters.
- The subscript e denotes extracellular concentrations.
- The subscript m denotes medium concentration.

Analyzing our Boolean network

Now, we need to find the fixed point(s) for all six possible parameter vectors, (G_e, L_e, L_{em}).

We can disregard the two cases where $L_e = 1$ and $L_{em} = 0$.

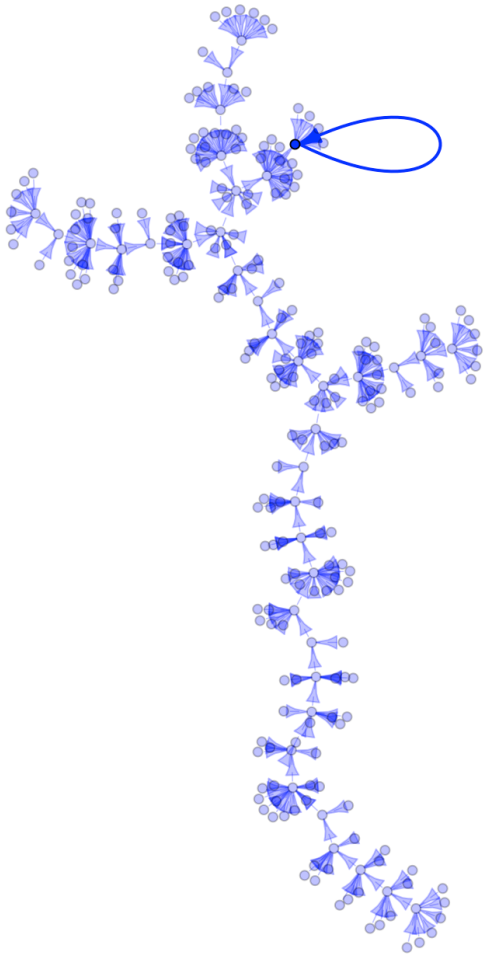
There are several (freely available) ways we can analyze systems like this:

- Use the BoolNet package in R to compute the fixed points, limit cycles, or plot the phase space. (Lots of additional capabilities.)
- Use a computer algebra package (Macaulay2, Singular, Sage) to convert the functions into polynomials, and compute the fixed points using Gröbner bases.
- Use Cyclone to nicely visualize the phase space with the nodes labeled as Boolean strings.
- Use GINsim (Gene Interaction Network simulation) to compute the fixed points and visualize the phase space.

All of these have their advantages and disadvantages.

Fixed point analysis and bistability

Here is the phase space with $(G_e, L_e, L_{em}) = (0, 0, 1)$, generated with BoolNet.



```
> print(getBasinOfAttraction(lacAttractorsBistable,2))
     State      Next state  Attr. basin  # trans. to attr.
1101001001 =>   1111000101            2                  1
1111001001 =>   1111000101            2                  1
1101000101 =>   1111000101            2                  1
1111000101 =>   1111000101            2                  0
1101001101 =>   1111000101            2                  1
1111001101 =>   1111000101            2                  1

Genes are encoded in the following order: M P B C R Rm A Am L Lm
```

Fixed point analysis and bistability

Computing the fixed point(s) for the other 5 initial conditions is an easy task for a computer.

(G_e, L_e, L_{em})	M	B	P	C	R	R_m	A	A_m	L	L_m	operon
$(1,0,0)$ $(1,0,1)$ $(1,1,1)$	0	0	0	0	1	1	0	0	0	0	OFF
$(0,0,0)$	0	0	0	1	1	1	0	0	0	0	OFF
$(0,1,1)$	1	1	1	1	0	0	1	1	1	1	ON
$(0,0,1)$	0 1	0 1	0 1	1 1	1 0	1 0	0 0	0 1	0 0	0 1	OFF ON

Suppose glucose or lactose are both absent ($L_e = L_{em} = G_e = 0$), so the operon is OFF:

$$(M, P, B, C, R, R_m, A, A_m, L, L_m) = (0,0,0,1,1,1,0,0,0,0).$$

Now, let's change L_{em} from 0 to 1, increasing lactose to medium. This is in the basin of the "low" bistable fixed point, so the operon remains OFF.

Conversely, suppose lactose concentration is high ($L_e = L_{em} = 1$), and so the operon is ON:

$$(M, P, B, C, R, R_m, A, A_m, L, L_m) = (1,1,1,1,0,0,0,1,0,1).$$

Now, let's change L_e from 1 to 0, reducing lactose levels to medium. This is in the basin of the "high" bistable fixed point, so the operon remains ON.

Time delay of activation

Suppose a protein A activates production of X, delayed by time τ (n steps).

We can introduce new "time-keeping" variables $A_1, A_2, \ldots, A_{n-1}$, with transition functions:

$$
\begin{aligned}
A_1(t+1) &= A(t) \\
A_2(t+1) &= A_1(t) \\
A_3(t+1) &= A_2(t) \\
&\vdots \\
A_{n-1}(t+1) &= A_{n-2}(t) \\
X(t+1) &= A_{n-1}(t)
\end{aligned}
$$

"X off; A switches on"

	A	A_1	A_2	A_3	A_4	X
$t=0$	1	0	0	0	0	0
$t=1$	1	1	0	0	0	0
$t=2$	1	1	1	0	0	0
$t=3$	1	1	1	1	0	0
$t=4$	1	1	1	1	1	0
$t=5$	1	1	1	1	1	1

"$n = 5$ seconds later, X turns on"

"X on; A switches off"

	A	A_1	A_2	A_3	A_4	X
$t=0$	0	1	1	1	1	1
$t=1$	0	0	1	1	1	1
$t=2$	0	0	0	1	1	1
$t=3$	0	0	0	0	1	1
$t=4$	0	0	0	0	0	1
$t=5$	0	0	0	0	0	0

"$n = 5$ seconds later, X turns off"

Though this increases the size of the state space, we can disregard "most" of the states.

For example, any global state with $(A_1, A_2, A_3, A_4) = (1, 0, 1, 0)$ is nonsensical.

One downside: it may not be realistic to assume that A toggling $0 \to 1$ vs. $1 \to 0$ will have the same time delay.

Time delay of activation

Suppose a protein A activates production of X, delayed by time τ (n steps).

But now, once A is shut off, X should be off the next timestep due to degredation.

This can be modeled by changing the update functions as shown.

$$
\begin{aligned}
A_1(t+1) &= A(t) \\
A_2(t+1) &= A_1(t) \wedge A(t) \\
A_3(t+1) &= A_2(t) \wedge A(t) \\
&\vdots \\
A_{n-1}(t+1) &= A_{n-2}(t) \wedge A(t) \\
X(t+1) &= A_{n-1}(t) \wedge A(t)
\end{aligned}
$$

"X off; A switches on"

	A	A_1	A_2	A_3	A_4	X
$t=0$	1	0	0	0	0	0
$t=1$	1	1	0	0	0	0
$t=2$	1	1	1	0	0	0
$t=3$	1	1	1	1	0	0
$t=4$	1	1	1	1	1	0
$t=5$	1	1	1	1	1	1

"$n = 5$ seconds later, X turns on"

"X on; A switches off"

	A	A_1	A_2	A_3	A_4	X
$t=0$	0	1	1	1	1	1
$t=1$	0	0	0	0	0	0

"$n = 1$ seconds later, X turns off"

Though this increases the size of the state space, we can disregard "most" of the states.

For example, $(A_1, A_2, A_3, A_4) = (1, 0, 1, 0)$ is nonsensical.

Time delay of inhibition

Let's repeat this, but now a protein R inhibits production of X, in one timestep.

However, once R is removed, then X will return, but delayed by time τ (n steps).

This can be modeled by changing the update functions as shown.

$$
\begin{aligned}
R_1(t+1) &= \overline{R(t)} \\
R_2(t+1) &= R_1(t) \wedge \overline{R(t)} \\
R_3(t+1) &= R_2(t) \wedge \overline{R(t)} \\
&\vdots \\
R_{n-1}(t+1) &= R_{n-2}(t) \wedge \overline{R(t)} \\
X(t+1) &= R_{n-1}(t) \wedge \overline{R(t)}
\end{aligned}
$$

"X off; R switches off"

	R	R_1	R_2	R_3	R_4	X
$t=0$	0	0	0	0	0	0
$t=1$	0	1	0	0	0	0
$t=2$	0	1	1	0	0	0
$t=3$	0	1	1	1	0	0
$t=4$	0	1	1	1	1	0
$t=5$	0	1	1	1	1	1

"$n=5$ seconds later, X turns on"

"X on; R switches on"

time	R	R_1	R_2	R_3	R_4	X
$t=0$	1	0	0	0	0	1
$t=1$	1	0	0	0	0	0

"$n=1$ seconds later, X turns off"

If we wanted both $0 \to 1$ and $1 \to 0$ time delays to be n steps, we could replace each

$$R_{i+1}(t+1) = R_i(t) \wedge \overline{R(t)} \qquad \text{with} \qquad R_{i+1}(t+1) = R_i(t).$$

Dilution and degradation

Suppose A regulates the production of X in 1 step: $A(t) = 1$ implies $X(t+1) = 1$.

Suppose that the loss of X due to dilution and degradation takes n timesteps.

We can model this by introducing new variables $X_1^{\downarrow}, X_2^{\downarrow}, \ldots, X_{n-1}^{\downarrow}$.

Properties

(i) If $Y(t) = 0$ and $X(t) = 1$, then $X_1^{\downarrow}(t+1) = 1$. (*"$X$ has been reduced once by dilution & degradation."*)

(ii) If $Y(t) = 0$ and $X_{i-1}^{\downarrow}(t) = 1$, then $X_i^{\downarrow}(t+1) = 1$. (*"$X$ has been reduced i times by dilution & degradation."*)

(iii) The number of "decay variables" is determined by the number of timesteps required to reduce $[X]$ below the discretation threshold.

Thus, $X(t+1) = 1$ when either of the following holds:

- $Y(t) = 1$ (new amount will be produced by $t+1$),
- $X(t) \wedge \overline{X_{n-1}^{\downarrow}(t)} = 1$ (previous amounts of X still available).

$$X(t+1) = Y(t) \vee \left(X(t) \wedge \overline{X_n^{\downarrow}(t)} \right)$$

Dilution and degradation

Let's now see an explicit example of this. Suppose that:

- A regulates the production of X in 1 step: $A(t) = 1$ implies $X(t+1) = 1$.
- the loss of X due to dilution and degradation takes n timesteps.

$$
\begin{aligned}
X_1^{\downarrow}(t+1) &= \overline{A(t)} \wedge X(t) \\
X_2^{\downarrow}(t+1) &= \overline{A(t)} \wedge X_1^{\downarrow}(t) \\
X_3^{\downarrow}(t+1) &= \overline{A(t)} \wedge X_2^{\downarrow}(t) \\
&\vdots \\
X_{n-1}^{\downarrow}(t+1) &= \overline{A(t)} \wedge X_{n-2}^{\downarrow}(t) \\
X(t+1) &= A(t) \vee \left[X(t) \wedge \overline{X_{n-1}^{\downarrow}(t)} \right]
\end{aligned}
$$

"X off; A switches on"

	A	$X_1^{\downarrow}$	$X_2^{\downarrow}$	$X_3^{\downarrow}$	$X_4^{\downarrow}$	X
$t=0$	1	0	0	0	0	0
$t=1$	1	0	0	0	0	1

"X on; A switches off"

	A	$X_1^{\downarrow}$	$X_2^{\downarrow}$	$X_3^{\downarrow}$	$X_4^{\downarrow}$	X
$t=0$	0	0	0	0	0	1
$t=1$	0	1	0	0	0	1
$t=2$	0	1	1	0	0	1
$t=3$	0	1	1	1	0	1
$t=4$	0	1	1	1	1	1
$t=5$	0	1	1	1	1	0

"$n = 5$ seconds later, X turns off"

Once again, this increases the state space size, but we can disregard "most" states.

For example, $(X_1^{\downarrow}, X_2^{\downarrow}, X_3^{\downarrow}, X_4^{\downarrow}) = (1, 0, 1, 0)$ is nonsensical.

In some sense, dilution and degradation are "dual" to time delays of activiation and inhibition.

Estimating constants for our Boolean model

3-variable ODE model of the *lac* operon (Yildirim and Mackey, 2004)

Let $M(t) =$ mRNA, $B(t) = \beta$-galactosidase, and $A(t) =$ allolactose (concentrations), respectively.

$$\frac{dM}{dt} = \alpha_M \frac{1 + K_1(e^{-\mu\tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu\tau_M} A_{\tau_M})^n} - \widetilde{\gamma_M} M$$

$$\frac{dB}{dt} = \alpha_B e^{-\mu\tau_B} M_{\tau_B} - \widetilde{\gamma_B} B$$

$$\frac{dA}{dt} = \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \widetilde{\gamma_A} A$$

We need to estimate these rate constants and time delays from the literature.

- Time delays: $\tau_M = .10$ min, $\tau_B = 2.00$ min.
- Degradation rates are harder to determine experimentally, and they vary widely in the literature. Sample values:

$$\begin{cases} \gamma_A = .52 \text{ min}^{-1}, \quad .0135 \text{ min}^{-1}, \quad .00018 \text{ min}^{-1} \\ \gamma_B = .00083 \text{ min}^{-1}, \\ \gamma_M = .411 \text{ min}^{-1}, \\ \mu \in (.0045,\ .0347) \end{cases}$$

Estimating constants for our Boolean model

Approach

We'll select "middle of range" estimates for the rate constants:

- $\mu = .03\ \text{min}^{-1}$,
- $\gamma_A = .014\ \text{min}^{-1} \implies \widetilde{\gamma_A} = \gamma_A + \mu = .044$,
- $\gamma_B = .001\ \text{min}^{-1} \implies \widetilde{\gamma_B} = \gamma_B + \mu = .031$,
- $\gamma_M = .411\ \text{min}^{-1} \implies \widetilde{\gamma_M} = \gamma_M + \mu = .441$.

Degradation is assumed to be exponential decay: $x' = -kx$ implies $x(t) = Ce^{-kt}$.

The half-life is the time t such that:

$$x(t) = Ce^{-kt} = .5C \implies e^{-kt} = .5 \implies -kt = \ln \tfrac{1}{2} \implies \boxed{t = \frac{\ln 2}{k}}$$

Half-lives

- $\widetilde{h_A} = \frac{\ln 2}{\widetilde{\gamma_A}} = 15.753$ (*approx. 1 time-step to decay*)
- $\widetilde{h_B} = \frac{\ln 2}{\widetilde{\gamma_B}} = 22.360$ (*approx. 2 time-steps to decay*)
- $\widetilde{h_M} = \frac{\ln 2}{\widetilde{\gamma_M}} = 1.5$ (*approx. 0 time-steps to decay*)

A Boolean model incorporating dilution and degradation

Model assumptions

- Variables are M, B, A.
- Glucose absent. Intracellular lactose present, two parameters: L and L_m.
- Time-step $\approx$ 12 min.
- *Ignore* (all $\ll 12$): $\tau_M = .10$ min, $\tau_B = 2$ min, $\widetilde{h_M} = 1.572$ min.
- Introduce variables for dilution and degradation:
  - $A_1^{\downarrow}$ (since $\widetilde{h_A} \approx 15.8 \approx 1$ timestep)
  - $B_1^{\downarrow}$, $B_2^{\downarrow}$ (since $\widetilde{h_B} \approx 22.4 \approx 2$ timesteps)

Proposed model

$$f_M = A \qquad f_B = M \vee \left(B \wedge \overline{B}_2^{\downarrow}\right)$$

$$f_A = (B \wedge L_m) \vee L \vee \left(A \wedge \overline{A}_1^{\downarrow} \wedge \overline{B}\right) \qquad f_{B_1^{\downarrow}} = \overline{M} \wedge B$$

$$f_{A_1^{\downarrow}} = \left((\overline{B} \vee \overline{L_m}) \wedge \overline{L}\right) \wedge A \qquad f_{B_2^{\downarrow}} = \overline{M} \wedge B_1^{\downarrow}$$

Most of the functions should be self-explanatory.

A Boolean model incorporating dilution and degradation

Justification for f_A

$$f_A = (B \wedge L_m) \vee L \vee \left(A \wedge \overline{A}_1^{\downarrow} \wedge \overline{B}\right)$$

There are 3 ways for allolactose to be available at $t+1$:

(i) β-galactosidase and at least medium levels of lactose are present;

(ii) high levels of lactose (assume basal concentrations of β-galactosidase);

(iii) Enough allolactose is present so that it's not degraded below the threshold, *and* no β-galactosidase is present.

Let's write our model into polynomials form, with parameters (L, L_m) and variables $(x_1, x_2, x_3, x_4, x_5, x_6) = (M, A, A_1^{\downarrow}, B, B_1^{\downarrow}, B_2^{\downarrow})$:

$$f_M = A$$
$$f_A = (B \wedge L_m) \vee L \vee \left(A \wedge A_1^{\downarrow} \wedge \overline{B}\right)$$
$$f_{A_1^{\downarrow}} = \left((\overline{B} \vee \overline{L_m}) \wedge \overline{L}\right) \wedge A$$
$$f_B = M \vee \left(B \wedge \overline{B}_2^{\downarrow}\right)$$
$$f_{B_1^{\downarrow}} = \overline{M} \wedge B$$
$$f_{B_2^{\downarrow}} = \overline{M} \wedge B_1^{\downarrow}$$

$$f_1 = x_2$$
$$f_2 = x_2(1+x_3)(1+x_4) + (L_m x_4 + L + x_4 L L_m) + x_2(1+x_3)(1+x_4)(L_m x_4 + L + x_4 L L_m)$$
$$f_3 = (1 + x_4 L_m)(1 + L)x_2$$
$$f_4 = x_1 + x_4(1 + x_6) + x_1 x_4(1 + x_6)$$
$$f_5 = (1 + x_1)x_4$$
$$f_6 = (1 + x_1)x_5$$

Using Macaulay2 to compute the fixed points (low lactose)

```
R = ZZ/2[M,A,A1,B,B1,B2];
I = ideal(M^2-M,A^2-A,A1^2-A1,B^2-B,B1^2-B1,B2^2-B2);
Q = R/I;

RingElement | RingElement :=(x,y)->x+y+x*y;
RingElement & RingElement :=(x,y)->x*y;

L = 0_Q; Lm = 0_Q;

fM = A;
fA = (B & Lm) | L | (A & (1+A1) & (1+B));
fA1 = (((1+B) | (1+Lm)) & (1+L)) & A;
fB = M | (B & (1+B2));
fB1 = (1+M) & B;
fB2 = (1+M) & B1;

I = ideal(fM+M, fA+A, fA1+A1, fB+B, fB1+B1, fB2+B2)
G = gens gb I
```

Output: `(B2 B1 B A1 A M)`

Conclusion: We have $B_2^{\downarrow} = B_1^{\downarrow} = B = A_1^{\downarrow} = A = M = 0$. There is a unique fixed point,

$$(M, A, A_1^{\downarrow}, B, B_1^{\downarrow}, B_2^{\downarrow}) = (0, 0, 0, 0, 0, 0).$$

This is exactly what we expect: the operon is OFF.

Using Macaulay2 to compute the fixed points (high lactose)

```
R = ZZ/2[M,A,A1,B,B1,B2];
I = ideal(M^2-M,A^2-A,A1^2-A1,B^2-B,B1^2-B1,B2^2-B2);
Q = R/I;

RingElement | RingElement :=(x,y)->x+y+x*y;
RingElement & RingElement :=(x,y)->x*y;

L = 1_Q; Lm = 1_Q;

fM = A;
fA = (B & Lm) | L | (A & (1+A1) & (1+B));
fA1 = (((1+B) | (1+Lm)) & (1+L)) & A;
fB = M | (B & (1+B2));
fB1 = (1+M) & B;
fB2 = (1+M) & B1;

I = ideal(fM+M, fA+A, fA1+A1, fB+B, fB1+B1, fB2+B2)
G = gens gb I
```

Output: `(B2  B1  B+1  A1  A+1  M+1)`

Conclusion: We have $B_2^{\downarrow} = B_1^{\downarrow} = A_1^{\downarrow}$ and $B = A = M = 1$. There is a unique fixed point,

$$(M, A, A_1^{\downarrow}, B, B_1^{\downarrow}, B_2^{\downarrow}) = (0, 0, 0, 0, 0, 0).$$

This is exactly what we expect: the operon is OFF.

Using Macaulay2 to compute the fixed points (medium lactose)

```
R = ZZ/2[M,A,A1,B,B1,B2];
I = ideal(M^2-M,A^2-A,A1^2-A1,B^2-B,B1^2-B1,B2^2-B2);
Q = R/I;

RingElement | RingElement :=(x,y)->x+y+x*y;
RingElement & RingElement :=(x,y)->x*y;

L = 0_Q; Lm = 1_Q;

fM = A;
fA = (B & Lm) | L | (A & (1+A1) & (1+B));
fA1 = (((1+B) | (1+Lm)) & (1+L)) & A;
fB = M | (B & (1+B2));
fB1 = (1+M) & B;
fB2 = (1+M) & B1;

I = ideal(fM+M, fA+A, fA1+A1, fB+B, fB1+B1, fB2+B2)
G = gens gb I
```

Output: `(B2  B1  A1  A+B  M+B)`

Conclusion: We have $B_2^{\downarrow} = B_1^{\downarrow} = A_1^{\downarrow} = 0$, and $A = B = M$. There are two fixed points,

$$(M, A, A_1^{\downarrow}, B, B_1^{\downarrow}, B_2^{\downarrow}) = (0,0,0,0,0,0), \quad \text{and} \quad (1,1,0,1,0,0).$$

In this case, the *lac* operon exhibits bistability.

Fixed points of our model and bistability

Here is a table showing the fixed points of our model, depending on whether extracellular lactose levels are low, medium, or high.

Inducer level	L	L_m	M	A	$A_1^{\downarrow}$	B	$B_1^{\downarrow}$	$B_2^{\downarrow}$	operon
Low lactose	0	0	0	0	0	0	0	0	OFF
High lactose	1	1	1	1	0	1	0	0	ON
Medium lactose	0	1	0	0	0	0	0	0	OFF
Medium lactose	0	1	1	1	0	1	0	0	ON

Suppose lactose concentration is low ($L = L_m = 0$), and so the operon is OFF. The current state is

$$(M, A, A_1^{\downarrow}, B, B_1^{\downarrow}, B_2^{\downarrow}) = (0, 0, 0, 0, 0, 0).$$

Now, let's change L_m from 0 to 1, increasing the lactose level to medium. We are now in the 3rd fixed point above, and so the operon is still OFF.

Conversely, suppose lactose concentration is high ($L = L_m = 1$), and so the operon is ON. The current state is

$$(M, A, A_1^{\downarrow}, B, B_1^{\downarrow}, B_2^{\downarrow}) = (1, 1, 0, 1, 0, 0).$$

Now, let's change L from 1 to 0, reducing the lactose level to medium. This takes us to the 4th fixed point above, and so the operon is still ON.

A Boolean model incorporating dilution & degradation, and time-delays

Instead of the a "middle value" (.0135 min^{-1}), let's choose the high estimate $\gamma_A = .52$ min^{-1}.

This makes the half-life of A (which was $\widetilde{h_A} = 15.753$) much smaller:

$$\widetilde{h_A} = \frac{\ln 2}{\widetilde{\gamma_A}} = 1.260, \qquad \widetilde{h_B} = \frac{\ln 2}{\widetilde{\gamma_B}} = 22.360 \qquad \widetilde{h_M} = \frac{\ln 2}{\widetilde{\gamma_M}} = 1.5$$

In this case, let's choose a much smaller time-step (e.g., $t = 1$ min).

We can no longer ignore all of the time-delays, so we introduce the following new variables:

- M_1, M_2 to model the delayed effect (by $\tau_B = 2$ min) of mRNA on the production of β-galactosidase.
- A_1 to model the delayed action of A on the production of mRNA by $\tau_M = .1$ min.

We will use the following new variables to model dilution & degradation:

- $M_1^{\downarrow}$ since $\widetilde{h_M} = 1.5$ is approximately 1 time-step.
- $A_1^{\downarrow}$ since $\widetilde{h_A} = 1.26$ is approximately 1 time-step.
- $B_1^{\downarrow}$, $B_2^{\downarrow}$ since loss of β-galactosidase is slower.

Remark

We really should use more variables, e.g., $B_1^{\downarrow}, B_2^{\downarrow}, \ldots, B_{22}^{\downarrow}$ to accurately track the loss of β-galactosidase. However, we will argue shortly why this won't matter.

A Boolean model incorporating dilution & degradation, and time-delays

Proposed model

$$f_M = A_1 \vee (M \wedge \overline{M}_1^{\downarrow}) \qquad f_{A_1} = A$$
$$f_{M_1} = M \qquad f_{A_1^{\downarrow}} = \left((\overline{B} \vee \overline{L_m}) \wedge \overline{L}\right) \wedge A$$
$$f_{M_2} = M_1 \wedge M \qquad f_B = M_2 \vee \left(B \wedge \overline{B}_2^{\downarrow}\right)$$
$$f_{M_1^{\downarrow}} = \overline{A_1} \wedge M \qquad f_{B_1^{\downarrow}} = \overline{M_2} \wedge B$$
$$f_A = (B \wedge L_m) \vee L \vee (A \wedge \overline{A}_1^{\downarrow} \wedge \overline{B}) \qquad f_{B_2^{\downarrow}} = \overline{M_2} \wedge B_1^{\downarrow}$$

Analysis of the long-term behavior of this model leads to similar results as the previous one.

Lactose	L	L_m	M	M_1	M_2	$M_1^{\downarrow}$	B	$B_1^{\downarrow}$	$B_2^{\downarrow}$	A	A_1	$A_1^{\downarrow}$
Low	0	0	0	0	0	0	0	0	0	0	0	0
High	1	1	1	1	1	0	1	0	0	1	1	0
Medium	0	1	0	0	0	0	0	0	0	0	0	0
Medium	0	1	1	1	1	0	1	0	0	1	1	0

A Boolean version of the 5-variable ODE model

5-variable ODE model (Yildirim and Mackey, 2004)

Let $M(t)$ = mRNA, $B(t)$ = β-galactosidase, $A(t)$ = allolactose, $P(t)$ = *lac* permease, $L(t)$ = lactose (concentrations). Extracellular lactose (L_e) is a parameter.

$$\begin{aligned}
\frac{dM}{dt} &= \alpha_M \frac{1 + K_1(e^{-\mu\tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu\tau_M} A_{\tau_M})^n} + \Gamma_0 - \widetilde{\gamma_M} M \\
\frac{dB}{dt} &= \alpha_B e^{-\mu\tau_B} M_{\tau_B} - \widetilde{\gamma_B} B \\
\frac{dA}{dt} &= \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \widetilde{\gamma_A} A \\
\frac{dP}{dt} &= \alpha_P e^{-\mu(\tau_B + \tau_P)} M_{\tau_B + \tau_P} - \widetilde{\gamma_P} P \\
\frac{dL}{dt} &= \alpha_L P \frac{L_e}{K_{L_e} + L_e} - \beta_{L_e} P \frac{L}{K_{L_e} + L} - \alpha_A B \frac{L}{K_L + L} - \widetilde{\gamma_L} L
\end{aligned}$$

We'll use the same estimates for degradation and delay constants as in the 3-variable model:

$$\mu = .03 \text{ min}^{-1}, \qquad \widetilde{\gamma_A} = \gamma + \mu = .044, \qquad \widetilde{\gamma_B} = \gamma + \mu = .031, \qquad \widetilde{\gamma_M} = \gamma + \mu = .441.$$

New degradation constants estimated at $\gamma_L = 0.0 \text{ min}^{-1}$, and $\gamma_P = .65 \text{ min}^{-1}$. Delay constant estimate is $\tau_P = .83$ min.

We need a new parameter to help distinguish high vs. medium extracellular lactose: L_{em}.

A Boolean version of the 5-variable ODE model

Model assumptions

- Variables are M, B, A, P, L.
- Glucose absent. Extracellular lactose present, two parameters: L_e and L_{em}.
- Ignore time-delays (Yildirim and Mackey showed that they do not affect bistability).
- Time-step $\approx$ 12 min.
- *Ignore* (all $\ll 12$): $\tau_M = .10$ min, $\tau_B = 2$ min, $\widetilde{h_M} = 1.572$ min.
- Introduce dilution & degradation variables: $A_1^{\downarrow}$, $B_1^{\downarrow}$, $L_1^{\downarrow}$, $P_1^{\downarrow}$.

Proposed model

$$f_M = A \vee (M \wedge \overline{M}_1) \qquad f_B = M \vee \left(B \wedge \overline{B}_1^{\downarrow}\right)$$
$$f_{M_1^{\downarrow}} = \overline{A} \wedge M \qquad f_{B_1^{\downarrow}} = \overline{M} \wedge B$$
$$f_A = (B \wedge L) \vee (L \wedge L_e) \vee \left(A \wedge \overline{A}_1^{\downarrow} \wedge \overline{B}\right) \qquad f_P = M \vee \left(P \wedge \overline{P}_1^{\downarrow}\right)$$
$$f_{A_1^{\downarrow}} = \left(\overline{B} \vee \overline{L}\right) \wedge \left(\overline{L} \vee \overline{L_e}\right) \wedge A \qquad f_{P_1^{\downarrow}} = \overline{M} \wedge P$$
$$f_L = ((P \wedge L_{em}) \vee L_e) \vee \left((L \wedge \overline{L}_1^{\downarrow}) \wedge (\overline{B} \wedge \overline{P})\right) \qquad f_{L_1^{\downarrow}} = \left((\overline{P} \vee \overline{L_{em}}) \wedge \overline{L_e}\right) \wedge L$$

A Boolean model incorporating dilution and degradation

Justification for f_A

$$f_A = (B \wedge L) \vee (L \wedge L_e) \vee (A \wedge \overline{A}_1^{\downarrow} \wedge \overline{B})$$

There are 3 ways for allolactose to be available at $t+1$:

(i) β-galactosidase and lactose are present.

(ii) Internal lactose is present and the concentration of extracellular lacatose is high. This ensures that by time $t+1$, intracellular lactose concentration is high enough to find available trace amounts of β-galactosidase.

(iii) The concentration of allolactose is high enough that it won't be reduced below the threshold due to dilution & degradation, or to conversion (by β-galactosidase) to glucose & galctose.

Justification for f_L

$$f_L = \left((P \wedge L_{em}) \vee L_e\right) \vee \left((L \wedge \overline{L}_1^{\downarrow}) \wedge (\overline{B} \wedge \overline{P})\right)$$

There are 3 ways for intracellular lactose to be available at $t+1$:

(i) *Lac* permease and extracellular lactose are available.

(ii) There are high levels of extracellular lactose available (even if *lac* permease level is low).

(iii) There is enough lactose in the cell that it won't be lost to dilution & degradaton, transport out, or conversion into allolactose (by β-galactosidase).

A Boolean model incorporating dilution and degradation

Model:

$$f_M = A \vee (M \wedge \overline{M}_1^{\downarrow}) \qquad f_B = M \vee \left(B \wedge \overline{B}_1^{\downarrow}\right)$$
$$f_{M_1^{\downarrow}} = \overline{A} \wedge M \qquad f_{B_1^{\downarrow}} = \overline{M} \wedge B$$
$$f_A = (B \wedge L) \vee (L \wedge L_e) \vee \left(A \wedge \overline{A}_1^{\downarrow} \wedge \overline{B}\right) \qquad f_P = M \vee \left(P \wedge \overline{P}_1^{\downarrow}\right)$$
$$f_{A_1^{\downarrow}} = \left(\overline{B} \vee \overline{L}\right) \wedge \left(\overline{L} \vee \overline{L_e}\right) \wedge A \qquad f_{P_1^{\downarrow}} = \overline{M} \wedge P$$
$$f_L = ((P \wedge L_{em}) \vee L_e) \vee \left((L \wedge \overline{L}_1^{\downarrow}) \wedge (\overline{B} \wedge \overline{P})\right) \qquad f_{L_1^{\downarrow}} = \left((\overline{P} \vee \overline{L_{em}}) \wedge \overline{L_e}\right) \wedge L$$

Fixed points:

Ext. Lactose	L_e	L_{em}	M	$M_1^{\downarrow}$	B	$B_1^{\downarrow}$	A	$A_1^{\downarrow}$	L	$L_1^{\downarrow}$	P	$P_1^{\downarrow}$
Low	0	0	0	0	0	0	0	0	0	0	0	0
High	1	1	1	0	1	0	1	0	1	0	1	0
Medium	0	1	0	0	0	0	0	0	0	0	0	0
Medium	0	1	1	0	1	0	1	0	1	0	1	0