Bistability in Boolean network models

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Motivation

Weaknesses of previous Boolean models

- All processes take a single timestep.
- Only assumed high and low levels in intracellular lactose. Medium levels are needed for bistability to exist.
- No time-delays incorporated.

In this section, we'll see how to add these types of features to Boolean models.

In particular, we'll see how to incorporate features such as:

- loss of concentration due to dilution and degradation;
- time-delays due to cellular processes

Dilution and degradation

Suppose Y regulates the production of X.

Assume $Y(t) = 1$ implies $X(t+1) = 1$. (activation takes 1 step).

Generally, the loss of X due to dilution and degradation takes several steps.

Introduce new variables $X_{\text{old}(1)}, X_{\text{old}(2)}, \ldots, X_{\text{old}(n)}$.

Properties

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

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

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

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

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

$$X(t+1) = Y(t) \vee \left(X(t) \wedge \overline{X_{\text{old}(n)}(t)} \right)$$

Other features

Medium levels of lactose

Introduce a new variable L_{high} so that $L_{\text{high}} = 1$ implies $L = 1$.

- High lactose: $L = 1$, $L_{\text{high}} = 1$.
- Medium lactose: $L = 1$, $L_{\text{high}} = 0$.
- Low lactose: $L = 0$, $L_{\text{high}} = 0$.

We can ignore any state for which $L = 0$, $L_{\text{high}} = 1$.

Previously, we introduced a variable L_ℓ to denote "*at least low levels of lactose*," so $(L, L_\ell) = (1, 0)$ described "medium lactose." This is an equally valid way to acheive the same goal.

Time delays

Say R regulates production of X, delayed by time τ (n steps).

Introduce new variables $R_1, R_2, \ldots, R_n$, with transition functions:

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

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.
- Degradtion rates are harder to determine experimentally, and they vary widely in the literaure. 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:

- $\mu = .03 \text{ min}^{-1}$,
- $\gamma_A = .014 \text{ min}^{-1} \implies \widetilde{\gamma_A} = \gamma_1 + \mu = .044$,
- $\gamma_B = .001 \text{ min}^{-1} \implies \widetilde{\gamma_B} = \gamma_B + \mu = .031$,
- $\gamma_M = .411 \text{ min}^{-1} \implies \widetilde{\gamma_M} = \gamma_M + \mu = .441$.

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_{high}.
- Time-step $\approx$ 10 min.
- *Ignore* (all $\ll 10$): $\tau_M = .10$ min, $\tau_B = 2$ min, $\widetilde{h_M} = 1.572$ min.
- Introduce variables for dilution and degradation:
  - A_{old} (since $\widetilde{h_A} \approx 15.8 \approx 1$ timestep)
  - B_{old}, $B_{\text{old}(2)}$ (since $\widetilde{h_B} \approx 22.4 \approx 2$ timesteps)

Proposed model

$$f_M = A \qquad f_B = M \vee \left(B \wedge \overline{B_{\text{old}(2)}}\right)$$

$$f_A = (B \wedge L) \vee L_{\text{high}} \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right) \qquad f_{B_{\text{old}(1)}} = \overline{M} \wedge B$$

$$f_{A_{\text{old}}} = \left((\overline{B} \vee \overline{L}) \wedge \overline{L_{\text{high}}}\right) \wedge A \qquad f_{B_{\text{old}(2)}} = \overline{M} \wedge B_{\text{old}(1)}$$

Most of the functions should be self-explanatory.

A Boolean model incorporating dilution and degradation

Justification for f_A

$$f_A = (B \wedge L) \vee L_{\text{high}} \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right)$$

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

(i) β-galactosidase and 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_h) and variables $(x_1, x_2, x_3, x_4, x_5, x_6) = (M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)})$:

$$f_M = A$$
$$f_A = (B \wedge L) \vee L_{\text{high}} \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right)$$
$$f_{A_{\text{old}}} = \left((\overline{B} \vee \overline{L}) \wedge \overline{L_{\text{high}}}\right) \wedge A$$
$$f_B = M \vee \left(B \wedge \overline{B_{\text{old}(2)}}\right)$$
$$f_{B_{\text{old}(1)}} = \overline{M} \wedge B$$
$$f_{B_{\text{old}(2)}} = \overline{M} \wedge B_{\text{old}(1)}$$

$$f_1 = x_2$$
$$f_2 = x_2(1+x_3)(1+x_4) + (Lx_4 + L_h + x_4 L L_h) + x_2(1+x_3)(1+x_4)(Lx_4 + L_h + x_4 L L_h)$$
$$f_3 = (1 + x_4 L)(1 + L_h)x_2$$
$$f_4 = x_1 + x_4(1 + x_6) + x_1 x_4(1 + x_6)$$
$$f_5 = (1 + x_1)x_4$$
$$f_6 = (1 + x_1)x_5$$

Using Sage to compute the fixed points (high lactose)

```
P.<x1,x2,x3,x4,x5,x6> = PolynomialRing(GF(2), 6, order ='lex'); P
```

```
Multivariate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field of size 2
```

```
L=1;
Lh=1;
print "L =", L;
print "L_h =", Lh;
```

```
L = 1
L_h = 1
```

```
I = ideal(x1+x2, x2+(L*x4+Lh+x4*L*Lh)+(x2*(1+x3)*(1+x4))+(L*x4+Lh+x4*L*Lh)*(x2*(1+x3)*(1+x4)), x3+(1+x4*L)*
(1+Lh)*x2, x4+x1+x4*(1+x6)+x1*x4*(1+x6), x5+(1+x1)*x4, x6+(1+x1)*x5); I
```

```
Ideal (x1 + x2, x2 + 1, x3, x1*x4*x6 + x1*x4 + x1 + x4*x6, x1*x4 + x4 + x5, x1*x5 + x5 + x6) of Multivar
iate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field of size 2
```

```
B = I.groebner_basis(); B
```

```
[x1 + 1, x2 + 1, x3, x4 + 1, x5, x6]
```

Conclusion: There is a unique fixed point,

$$(M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)}) = (x_1, x_2, x_3, x_4, x_5, x_6) = (1, 1, 0, 1, 0, 0)$$

This is exactly what we expected: the *lac* operon is ON.

Using Sage to compute the fixed points (low lactose)

```
P.<x1,x2,x3,x4,x5,x6> = PolynomialRing(GF(2), 6, order ='lex'); P
```

```
Multivariate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field of size 2
```

```
L=0;
Lh=0;
print "L =", L;
print "L_h =", Lh;
```

```
L = 0
L_h = 0
```

```
I = ideal(x1+x2, x2+(L*x4+Lh+x4*L*Lh)+(x2*(1+x3)*(1+x4))+(L*x4+Lh+x4*L*Lh)*(x2*(1+x3)*(1+x4)), x3+(1+x4*L)*(1+Lh)*x2, x4+x1+x4*(1+x6)+x1*x4*(1+x6), x5+(1+x1)*x4, x6+(1+x1)*x5); I
```

```
Ideal (x1 + x2, x2*x3*x4 + x2*x3 + x2*x4, x2 + x3, x1*x4*x6 + x1*x4 + x1 + x4*x6, x1*x4 + x4 + x5, x1*x5 + x5 + x6) of Multivariate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field of size 2
```

```
B = I.groebner_basis(); B
```

```
[x1 + x6^2, x2 + x6^2, x3 + x6^2, x4 + x6^5 + x6^4 + x6, x5 + x6^4 + x6, x6^6 + x6^4 + x6^3]
```

We need to backsubstitute. Recall that $x_i^k = x_i$ for all k.

The last equation: $x_6^6 + x_6^4 + x_6^3 = 0$ implies $x_6 = 0$.

Plug this into the previous equation: $x_5 + x_6^4 + x_6 = 0$ (with $x_6 = 0$) implies $x_5 = 0$.

And so on. We get a unique fixed point:

$$(M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)}) = (x_1, x_2, x_3, x_4, x_5, x_6) = (0, 0, 0, 0, 0, 0)$$

This is exactly what we expected: the *lac* operon is OFF.

Using Sage to compute the fixed points (medium lactose)

```
P.<x1,x2,x3,x4,x5,x6> = PolynomialRing(GF(2), 6, order ='lex'); P
```

```
Multivariate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field of size 2
```

```
L=1;
Lh=0;
print "L =", L;
print "L_h =", Lh;
```

```
L = 1
L_h = 0
```

```
I = ideal(x1+x2, x2+(L*x4+Lh+x4*L*Lh)+(x2*(1+x3)*(1+x4))+(L*x4+Lh+x4*L*Lh)*(x2*(1+x3)*(1+x4)), x3+(1+x4*L)*(1+Lh)*x2, x4+x1+x4*(1+x6)+x1*x4*(1+x6), x5+(1+x1)*x4, x6+(1+x1)*x5); I
```

```
Ideal (x1 + x2, x2*x3*x4^2 + x2*x3 + x2*x4^2 + x4, x2*x4 + x2 + x3, x1*x4*x6 + x1*x4 + x1 + x4*x6, x1*x4 + x4 + x5, x1*x5 + x5 + x6) of Multivariate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field of size 2
```

```
B = I.groebner_basis(); B
```

```
[x1 + x4 + x6^9 + x6^8 + x6^5 + x6^4, x2 + x4 + x6^9 + x6^8 + x6^5 + x6^4, x3 + x6^9 + x6^5, x4^2 + x4 + x6^11 + x6^10 + x6^9 + x6^8 + x6^6, x4*x6 + x6^10 + x6^9 + x6^6 + x6^2, x5 + x6^8 + x6^4, x6^12 + x6^9 + x6^5 + x6^4 + x6]
```

The last (7th) equation implies $x_6 = 0$. The 6th one then implies $x_5 = 0$.

The 5th equation gives no information (x_4 can be anything), as does the 4th ($x_4^2 + x_4 = 0$).

The 3rd equation says $x_3 = 0$.

The 2nd equation says $x_2 = x_4$, and the 1st equation says $x_1 = x_4$.

We get two fixed points:

$$(M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)}) = (x_1, x_2, x_3, x_4, x_5, x_6) = (0,0,0,0,0,0), \text{ or } (1,1,0,1,0,0).$$

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_{high}	M	B	$B_{\text{old}(1)}$	$B_{\text{old}(2)}$	A	A_{old}	operon
Low lactose	0	0	0	0	0	0	0	0	OFF
High lactose	1	1	1	1	0	0	1	0	ON
Medium lactose	1	0	0	0	0	0	0	0	OFF
Medium lactose	1	0	1	1	0	0	1	0	ON

Suppose lactose concentration is low ($L = L_{\text{high}} = 0$), and so the operon is OFF. The current state is

$$(M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)}) = (x_1, x_2, x_3, x_4, x_5, x_6) = (0, 0, 0, 0, 0, 0),$$

Now, let's change L 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 ($L = L_{\text{high}} = 1$), and so the operon is ON. The current state is

$$(M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)}) = (x_1, x_2, x_3, x_4, x_5, x_6) = (1, 1, 0, 1, 0, 0),$$

Now, let's change L_{high} 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" ($.135\ \text{min}^{-1}$), let's choose the high estimate $\gamma_A = .52\ \text{min}^{-1}$.

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_{old} since $\widetilde{\gamma_M} = 1.5$ is approximately 1 time-step.
- A_{old} since $\widetilde{\gamma_A} = 1.26$ is approximately 1 time-step.
- $B_{\text{old}(1)}$, $B_{\text{old}(2)}$ since loss of β-galactosidase is slower.

Remark

We really should use more variables, e.g., $B_{\text{old}(1)}, B_{\text{old}(2)}, \ldots, B_{\text{old}(22)}$ 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

$$f_M = A_1 \vee (M \wedge \overline{M_{\text{old}}}) \qquad f_{A_1} = A$$
$$f_{M_1} = M \qquad f_{A_{\text{old}}} = \left((\overline{B} \vee \overline{L}) \wedge \overline{L_{\text{high}}}\right) \wedge A$$
$$f_{M_2} = M_1 \qquad f_B = M_2 \vee \left(B \wedge \overline{B_{\text{old}(2)}}\right)$$
$$f_{M_{\text{old}}} = \overline{A_1} \wedge M \qquad f_{B_{\text{old}(1)}} = \overline{M_2} \wedge B$$
$$f_A = (B \wedge L) \vee L_{\text{high}} \vee (A \wedge \overline{A_{\text{old}}} \wedge \overline{B}) \qquad f_{B_{\text{old}(2)}} = \overline{M_2} \wedge B_{\text{old}\,(1)}$$

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

Lactose	L	L_{high}	M	M_1	M_2	M_{old}	B	$B_{\text{old}(1)}$	$B_{\text{old}(2)}$	A	A_1	A_{old}
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	1	0	0	0	0	0	0	0	0	0	0	0
Medium	1	0	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: $L_{e_{\text{high}}}$.

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 $L_{e_{\text{high}}}$.
- Ignore time-delays (Yildirim and Mackey showed that they do not affect bistability).
- Time-step $\approx$ 10 min.
- *Ignore* (all $\ll 10$): $\tau_M = .10$ min, $\tau_B = 2$ min, $\widetilde{h_M} = 1.572$ min.
- Introduce dilution & degradation variables: A_{old}, B_{old}, L_{old}, P_{old}.

Proposed model

$$f_M = A \vee (M \wedge \overline{M_{\text{old}}}) \qquad f_B = M \vee \left(B \wedge \overline{B_{\text{old}}}\right)$$

$$f_{M_{\text{old}}} = \overline{A} \wedge M \qquad f_{B_{\text{old}}} = \overline{M} \wedge B$$

$$f_A = (B \wedge L) \vee (L \wedge L_{e_{\text{high}}}) \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right) \qquad f_P = M \vee \left(P \wedge \overline{P_{\text{old}}}\right)$$

$$f_{A_{\text{old}}} = \left(\overline{B} \vee \overline{L}\right) \wedge \left(\overline{L} \vee \overline{L_{e_{\text{high}}}}\right) \wedge A \qquad f_{P_{\text{old}}} = \overline{M} \wedge P$$

$$f_L = \left((P \wedge L_e) \vee L_{e_{\text{high}}}\right) \vee \left((L \wedge \overline{L_{\text{old}}}) \wedge (\overline{B} \wedge \overline{P})\right) \qquad f_{L_{\text{old}}} = \left((\overline{P} \vee \overline{L_e}) \wedge \overline{L_{e_{\text{high}}}}\right) \wedge L$$

A Boolean model incorporating dilution and degradation

Justification for f_A

$$f_A = (B \wedge L) \vee (L \wedge L_{e_{\text{high}}}) \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right)$$

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) 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 avaialbe trace amounts of β-galactosidase.

(iii) The concentration of allolactose is high enough that it wont' be reduced below the threshold due to dilution & degradation, or to conversion (by β-galactosidase) to glucose & galctose.

Justification for f_L

$$f_L = \left((P \wedge L_e) \vee L_{e_{\text{high}}}\right) \vee \left((L \wedge \overline{L_{\text{old}}}) \wedge (\overline{B} \wedge \overline{P})\right)$$

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:

$$f_M = A \vee (M \wedge \overline{M_{\text{old}}}) \qquad f_B = M \vee \left(B \wedge \overline{B_{\text{old}}}\right)$$
$$f_{M_{\text{old}}} = \overline{A} \wedge M \qquad f_{B_{\text{old}}} = \overline{M} \wedge B$$
$$f_A = (B \wedge L) \vee (L \wedge L_{e_{\text{high}}}) \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right) \qquad f_P = M \vee \left(P \wedge \overline{P_{\text{old}}}\right)$$
$$f_{A_{\text{old}}} = \left(\overline{B} \vee \overline{L}\right) \wedge \left(\overline{L} \vee \overline{L_{e_{\text{high}}}}\right) \wedge A \qquad f_{P_{\text{old}}} = \overline{M} \wedge P$$
$$f_L = \left((P \wedge L_e) \vee L_{e_{\text{high}}}\right) \vee \left((L \wedge \overline{L_{\text{old}}}) \wedge (\overline{B} \wedge \overline{P})\right) \qquad f_{L_{\text{old}}} = \left((\overline{P} \vee \overline{L_e}) \wedge \overline{L_{e_{\text{high}}}}\right) \wedge L$$

Fixed points:

Ext. Lactose	L_e	$L_{e_{\text{high}}}$	M	M_{old}	B	B_{old}	A	A_{old}	L	L_{old}	P	P_{old}
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	1	0	0	0	0	0	0	0	0	0	0	0
Medium	1	0	1	0	1	0	1	0	1	0	1	0