Dilution, degradation, and time delays in Boolean network models

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Algebraic Biology
Motivation

We’ve seen how to incorporate the following features into ODE models:

- dilution of protein concentration due to cellular growth;
- degradation (or decay) of protein concentration;
- time-delays due to cellular processes.

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

Our Boolean models will be derived from the 3-variable and 5-variable ODE models from the previous lecture.
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 \( n \) timesteps.

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

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 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 \( t + 1 \)),
- \( X(t) \land X_{\text{old}}(n-1)(t) = 1 \) (previous amounts of \( X \) still available).

\[
X(t + 1) = Y(t) \lor \left( X(t) \land X_{\text{old}}(n)(t) \right)
\]
Other features

Time delays

Say $R$ regulates production of $X$, delayed by time $\tau$ ($n$ steps).

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

\[
\begin{align*}
R_1(t + 1) &= R(t) \\
R_2(t + 1) &= R_1(t) \\
R_3(t + 1) &= R_2(t) \\
&\vdots \\
R_{n-1}(t + 1) &= R_{n-2}(t) \\
X(t + 1) &= R_n(t)
\end{align*}
\]

Medium levels of lactose

Introduce a new variable $L_m$ meaning “at least medium levels” of lactose. Clearly, $L = 1$ implies $L_m = 1$.

- 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 $L = 1, L_m = 0$. 
Estimating constants for our Boolean model

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

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

\[
\frac{dM}{dt} = \alpha_M \frac{1 + K_1(e^{-\mu \tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu \tau_M} A_{\tau_M})^n} - \gamma_M M
\]
\[
\frac{dB}{dt} = \alpha_B e^{-\mu \tau_B} M_{\tau_B} - \gamma_B B
\]
\[
\frac{dA}{dt} = \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \gamma_A A
\]

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

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

\[
\left\{
\begin{array}{ll}
    \gamma_A = .52 \text{ min}^{-1}, & .0135 \text{ min}^{-1}, & .00018 \text{ min}^{-1} \\
    \gamma_B = .00083 \text{ min}^{-1}, \\
    \gamma_M = .411 \text{ min}^{-1}, \\
    \mu \in (.0045, .0347)
\end{array}
\right.
\]
Estimating constants for our Boolean model

Approach

We’ll select “middle of range” estimates for the rate constants:

- \( \mu = 0.03 \text{ min}^{-1} \),
- \( \gamma_A = 0.014 \text{ min}^{-1} \Rightarrow \tilde{\gamma}_A = \gamma_A + \mu = 0.044 \),
- \( \gamma_B = 0.001 \text{ min}^{-1} \Rightarrow \tilde{\gamma}_B = \gamma_B + \mu = 0.031 \),
- \( \gamma_M = 0.411 \text{ min}^{-1} \Rightarrow \tilde{\gamma}_M = \gamma_M + \mu = 0.441 \).

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

The half-life is the time \( t \) such that:

\[
x(t) = Ce^{-kt} = 0.5C \quad \Rightarrow \quad e^{-kt} = 0.5 \quad \Rightarrow \quad -kt = \ln \frac{1}{2} \quad \Rightarrow \quad t = \frac{\ln 2}{k}
\]

Half-lives

- \( \tilde{h}_A = \frac{\ln 2}{\gamma_A} = 15.753 \) \( (\text{approx. 1 time-step to decay}) \)
- \( \tilde{h}_B = \frac{\ln 2}{\gamma_B} = 22.360 \) \( (\text{approx. 2 time-steps to decay}) \)
- \( \tilde{h}_M = \frac{\ln 2}{\gamma_M} = 1.5 \) \( (\text{approx. 0 time-steps to decay}) \)
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, $\tilde{h}_M = 1.572$ min.
- Introduce variables for dilution and degradation:
  - $A_{old}$ (since $\tilde{h}_A \approx 15.8 \approx 1$ timestep)
  - $B_{old}$, $B_{old(2)}$ (since $\tilde{h}_B \approx 22.4 \approx 2$ timesteps)

Proposed model

\[
\begin{align*}
    f_M &= A \\
    f_A &= (B \land L_m) \lor L \lor \left( A \land \overline{A_{old}} \land \overline{B} \right) \\
    f_{A_{old}} &= \left( \overline{B} \lor L_m \right) \land L \land A
\end{align*}
\]

\[
\begin{align*}
    f_B &= M \lor \left( B \land \overline{B_{old(2)}} \right) \\
    f_{B_{old(1)}} &= \overline{M} \land B \\
    f_{B_{old(2)}} &= \overline{M} \land B_{old(1)}
\end{align*}
\]

Most of the functions should be self-explanatory.
A Boolean model incorporating dilution and degradation

Justification for $f_A$

$$f_A = (B \land L_m) \lor L \lor \left( A \land \overline{A_{old}} \land \overline{B} \right)$$

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

(i) $\beta$-galactosidase and at least medium levels of lactose are present;
(ii) high levels of lactose (assume basal concentrations of $\beta$-galactosidase);
(iii) Enough allolactose is present so that it’s not degraded below the threshold, and no $\beta$-galactosidase is present.

Let’s write our model into polynomials form, with parameters $(L, L_m)$ and variables $(x_1, x_2, x_3, x_4, x_5, x_6) = (M, A, A_{old}, B, B_{old(1)}, B_{old(2)})$:

$$f_M = A$$
$$f_A = (B \land L_m) \lor L \lor \left( A \land \overline{A_{old}} \land \overline{B} \right)$$
$$f_{A_{old}} = \left( \overline{B} \lor L_m \land L \right) \land A$$
$$f_B = M \lor \left( B \land \overline{B_{old(2)}} \right)$$
$$f_{B_{old(1)}} = \overline{M} \land B$$
$$f_{B_{old(2)}} = \overline{M} \land B_{old(1)}$$

$$f_1 = x_2$$
$$f_2 = x_2(1+x_3)(1+x_4) + (L_m x_4 + L + x_4 LL_m) + x_2(1+x_3)(1+x_4)(L_m x_4 + L + x_4 LL_m)$$
$$f_3 = (1 + x_4 L_m)(1 + L)x_2$$
$$f_4 = x_1 + x_4(1 + x_6) + x_1 x_4(1 + x_6)$$
$$f_5 = (1 + x_1)x_4$$
$$f_6 = (1 + x_1)x_5$$
Using Sage to compute the fixed points (high lactose)

```python
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 Multivariate 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,

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

This is exactly what we expected: the lac operon is ON.
Using Sage to compute the fixed points (low lactose)

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)

```python
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*Lh)+(x2*(1+x3)*(1+x4))+(L*x4+Lh+x4*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.

<table>
<thead>
<tr>
<th>Inducer level</th>
<th>$L$</th>
<th>$L_m$</th>
<th>$M$</th>
<th>$A$</th>
<th>$A_{\text{old}}$</th>
<th>$B$</th>
<th>$B_{\text{old}(1)}$</th>
<th>$B_{\text{old}(2)}$</th>
<th>operon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low lactose</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>OFF</td>
</tr>
<tr>
<td>High lactose</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>ON</td>
</tr>
<tr>
<td>Medium lactose</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>OFF</td>
</tr>
<tr>
<td>Medium lactose</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>ON</td>
</tr>
</tbody>
</table>

Suppose lactose concentration is low ($L = L_m = 0$), 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 $L_m$ 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

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

Now, let’s change $L$ 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 $\tilde{h}_A = 15.753$) much smaller:

$$\tilde{h}_A = \frac{\ln 2}{\gamma_A} = 1.260, \quad \tilde{h}_B = \frac{\ln 2}{\gamma_B} = 22.360 \quad \tilde{h}_M = \frac{\ln 2}{\gamma_M} = 1.5$$

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 $\beta$-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:

- $M_{\text{old}}$ since $\tilde{h}_M = 1.5$ is approximately 1 time-step.
- $A_{\text{old}}$ since $\tilde{h}_A = 1.26$ is approximately 1 time-step.
- $B_{\text{old}(1)}, B_{\text{old}(2)}$ since loss of $\beta$-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 $\beta$-galactosidase. However, we will argue shortly why this won’t matter.
A Boolean model incorporating dilution & degradation, and time-delays

Proposed model

\[
\begin{align*}
  f_M &= A_1 \lor (M \land \overline{M_{\text{old}}}) \\
  f_{M_1} &= M \\
  f_{M_2} &= M_1 \\
  f_{M_{\text{old}}} &= \overline{A_1} \land M \\
  f_A &= (B \land L_m) \lor L \lor (A \land \overline{A_{\text{old}}} \land \overline{B})
\end{align*}
\]

\[
\begin{align*}
  f_{A_1} &= A \\
  f_{A_{\text{old}}} &= (\overline{B} \lor \overline{L_m}) \land \overline{L} \land A \\
  f_B &= M_2 \lor (B \land \overline{B_{\text{old}(2)}}) \\
  f_{B_{\text{old}(1)}} &= \overline{M_2} \land B \\
  f_{B_{\text{old}(2)}} &= \overline{M_2} \land B_{\text{old}(1)}
\end{align*}
\]

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

<table>
<thead>
<tr>
<th>Lactose</th>
<th>(L)</th>
<th>(L_m)</th>
<th>(M)</th>
<th>(M_1)</th>
<th>(M_2)</th>
<th>(M_{\text{old}})</th>
<th>(B)</th>
<th>(B_{\text{old}(1)})</th>
<th>(B_{\text{old}(2)})</th>
<th>(A)</th>
<th>(A_1)</th>
<th>(A_{\text{old}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>High</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
A Boolean version of the 5-variable ODE model

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

Let $M(t) = \text{mRNA}$, $B(t) = \beta$-galactosidase, $A(t) = \text{allo lac}$, $P(t) = \text{lac permease}$, $L(t) = \text{lactose (concentrations)}$. Extracellular lactose ($L_e$) is a parameter.

\[
\begin{align*}
\frac{dM}{dt} &= \alpha_M \frac{1 + K_1(e^{-\mu t M} A_{\tau M})^n}{K + K_1(e^{-\mu t M} A_{\tau M})^n} + \Gamma_0 - \gamma_M M \\
\frac{dB}{dt} &= \alpha_B e^{-\mu t B} M_{\tau B} - \gamma_B B \\
\frac{dA}{dt} &= \alpha_{AB} \frac{L}{K_L + L} - \beta_{AB} \frac{A}{K_A + A} - \gamma_A A \\
\frac{dP}{dt} &= \alpha_P e^{-\mu (\tau_B + \tau_P)} M_{\tau B + \tau_P} - \gamma_P P \\
\frac{dL}{dt} &= \alpha_L P \frac{L_e}{K_{Le} + L_e} - \beta_{Le} P \frac{L}{K_{Le} + L} - \alpha_{AB} \frac{L}{K_L + L} - \gamma_L L
\end{align*}
\]

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

\[
\begin{align*}
\mu &= .03 \text{ min}^{-1}, \quad \gamma_A = \gamma + \mu = .044, \quad \gamma_B = \gamma + \mu = .031, \quad \gamma_M = \gamma + \mu = .441.
\end{align*}
\]

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

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, \(\hat{h}_M = 1.572\) min.
- Introduce dilution & degradation variables: \(A_{old}, B_{old}, L_{old}, P_{old}\).

Proposed model

\[
\begin{align*}
 f_M &= A \lor (M \land M_{old}) \\
 f_{M_{old}} &= \overline{A} \land M \\
 f_A &= (B \land L) \lor (L \land L_e) \lor (A \land A_{old} \land \overline{B}) \\
 f_{A_{old}} &= (\overline{B} \lor \overline{L}) \land (\overline{L} \lor L_e) \land A \\
 f_L &= ((P \land L_{em}) \lor L_e) \lor ((L \land L_{old}) \land (\overline{B} \land \overline{P})) \\
 f_{L_{old}} &= (\overline{P} \lor L_{em}) \land L_e \land L
\end{align*}
\]
A Boolean model incorporating dilution and degradation

### Justification for $f_A$

$$f_A = (B \land L) \lor (L \land L_e) \lor \left( A \land \overline{A_{\text{old}}} \land \overline{B} \right)$$

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

(i) $\beta$-galactosidase and lactose are present.

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

### Justification for $f_L$

$$f_L = ((P \land L_{em}) \lor L_e) \lor \left( (L \land \overline{L_{\text{old}}}) \land (\overline{B} \land \overline{P}) \right)$$

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 & degradation, transport out, or conversion into allolactose (by $\beta$-galactosidase).
A Boolean model incorporating dilution and degradation

Model:

\[
\begin{align*}
 f_M &= A \lor (M \land \overline{M_{\text{old}}}) \\
 f_{M_{\text{old}}} &= \overline{A} \land M \\
 f_A &= (B \land L) \lor (L \land L_e) \lor (A \land \overline{A_{\text{old}}} \land \overline{B}) \\
 f_{A_{\text{old}}} &= (\overline{B} \lor \overline{L}) \land (\overline{L} \lor \overline{L_e}) \land A \\
 f_L &= ((P \land L_{\text{em}}) \lor L_e) \lor ((L \land \overline{L_{\text{old}}}) \land (\overline{B} \land \overline{P})) \\
 f_{L_{\text{old}}} &= ((\overline{P} \lor \overline{L_{\text{em}}}) \land \overline{L_e}) \land P \\
 f_B &= M \lor (B \land \overline{B_{\text{old}}}) \\
 f_{B_{\text{old}}} &= \overline{M} \land B \\
 f_P &= M \lor (P \land \overline{P_{\text{old}}}) \\
 f_{P_{\text{old}}} &= \overline{M} \land P
\end{align*}
\]

Fixed points:

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<th>(L_{\text{em}})</th>
<th>(M)</th>
<th>(M_{\text{old}})</th>
<th>(B)</th>
<th>(B_{\text{old}})</th>
<th>(A)</th>
<th>(A_{\text{old}})</th>
<th>(L)</th>
<th>(L_{\text{old}})</th>
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