

Dilution, degradation, and time delays in Boolean network models

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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 several steps.

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

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 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 $t + 1$),
- $X(t) \wedge \overline{X_{\text{old}(n)}(t)} = 1$ (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

Time delays

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

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

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

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: $L = 0, L_m = 1$.
- Basal 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 $M(t)$ = mRNA, $B(t)$ = β -galactosidase, and $A(t)$ = allolactose (concentrations), respectively.

$$\begin{aligned}\frac{dM}{dt} &= \alpha_M \frac{1 + K_1(e^{-\mu\tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu\tau_M} A_{\tau_M})^n} - \tilde{\gamma}_M M \\ \frac{dB}{dt} &= \alpha_B e^{-\mu\tau_B} M_{\tau_B} - \tilde{\gamma}_B B \\ \frac{dA}{dt} &= \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \tilde{\gamma}_A A\end{aligned}$$

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:

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

Estimating constants for our Boolean model

Approach

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

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

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

The **half-life** is the time t such that:

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

Half-lives

- $\tilde{h}_A = \frac{\ln 2}{\tilde{\gamma}_A} = 15.753$ (approx. 1 time-step to decay)
- $\tilde{h}_B = \frac{\ln 2}{\tilde{\gamma}_B} = 22.360$ (approx. 2 time-steps to decay)
- $\tilde{h}_M = \frac{\ln 2}{\tilde{\gamma}_M} = 1.5$ (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 ≈ 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)
 - $B_{\text{old}}, B_{\text{old}(2)}$ (since $\tilde{h}_B \approx 22.4 \approx 2$ timesteps)

Proposed model

$$f_M = A$$

$$f_A = (B \wedge L_m) \vee L \vee (A \wedge \overline{A_{\text{old}}} \wedge \overline{B})$$

$$f_{A_{\text{old}}} = ((\overline{B} \vee \overline{L_m}) \wedge \overline{L}) \wedge A$$

$$f_B = M \vee (B \wedge \overline{B_{\text{old}(2)}})$$

$$f_{B_{\text{old}(1)}} = \overline{M} \wedge B$$

$$f_{B_{\text{old}(2)}} = \overline{M} \wedge B_{\text{old}(1)}$$

Most of the functions should be self-explanatory.

A Boolean model incorporating dilution and degradation

Justification for f_A

$$f_A = (B \wedge L_m) \vee L \vee (A \wedge \overline{A_{\text{old}}} \wedge \overline{B})$$

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

$$f_M = A$$

$$f_A = (B \wedge L_m) \vee L \vee (A \wedge \overline{A_{\text{old}}} \wedge \overline{B})$$

$$f_{A_{\text{old}}} = ((\overline{B} \vee \overline{L_m}) \wedge \overline{L}) \wedge A$$

$$f_B = M \vee (B \wedge \overline{B_{\text{old}(2)}})$$

$$f_{B_{\text{old}(1)}} = \overline{M} \wedge B$$

$$f_{B_{\text{old}(2)}} = \overline{M} \wedge B_{\text{old}(1)}$$

$$f_1 = x_2$$

$$f_2 = x_2(1+x_3)(1+x_4) + (L_m x_4 + L + x_4 L L_m) \\ + x_2(1+x_3)(1+x_4)(L_m x_4 + L + x_4 L L_m)$$

$$f_3 = (1 + x_4 L_m)(1 + L)x_2$$

$$f_4 = x_1 + x_4(1 + x_6) + x_1 x_4(1 + x_6)$$

$$f_5 = (1 + x_1)x_4$$

$$f_6 = (1 + x_1)x_5$$

Using Sage to compute the fixed points (high lactose)

```
1 |
2 | P.<x1,x2,x3,x4,x5,x6> = PolynomialRing(GF(2), 6, order = 'lex'); P
3 | Multivariate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field of size 2
4 |
5 | L=1;
6 | Lh=1;_
7 | print "L =", L;_
8 | print "L_h =", Lh;
9 |
10 | L = 1
11 | L_h = 1
12 | 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)*
13 | (1+Lh)*x2, x4+x1+x4*(1+x6)+x1*x4*(1+x6), x5+(1+x1)*x4, x6+(1+x1)*x5); I
14 | Ideal (x1 + x2, x2 + 1, x3, x1*x4*x6 + x1*x4 + x1 + x4*x6, x1*x4 + x4 + x5, x1*x5 + x5 + x6) of Multivar
15 | iate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field of size 2
16 |
17 | B = I.groebner_basis(); B
18 | [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)

```
1 |-----|
2 | P.<x1,x2,x3,x4,x5,x6> = PolynomialRing(GF(2), 6, order = 'lex'); P
3 | Multivariate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field of size 2
4 |-----|
5 | L=0;
6 | Lh=0;
7 | print "L =", L;
8 | print "L_h =", Lh;
9 |
10 | L = 0
11 | L_h = 0
12 |-----|
13 | 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)*
14 | (1+Lh)*x2, x4+x1+x4*(1+x6)+x1*x4*(1+x6), x5+(1+x1)*x4, x6+(1+x1)*x5); I
15 | 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
16 | + x5 + x6) of Multivariate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field of size 2
17 |-----|
18 | B = I.groebner_basis(); B
19 | [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)

```
1
2 P.<x1,x2,x3,x4,x5,x6> = PolynomialRing(GF(2), 6, order = 'lex'); P
3   Multivariate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field of size 2
4
5 L=1;
6 Lh=0;
7 print "L =", L;
8 print "L_h =", Lh;
9
10   L = 1
11   L_h = 0
12
13 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)*
14 (1+Lh)*x2, x4+x1+x4*(1+x6)+x1*x4*(1+x6), x5+(1+x1)*x4, x6+(1+x1)*x5); I
15
16   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
17   + x4 + x5, x1*x5 + x5 + x6) of Multivariate Polynomial Ring in x1, x2, x3, x4, x5, x6 over Finite Field
18   of size 2
19
20 B = I.groebner_basis(); B
21
22   [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 +
23   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
24   + 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_m	M	B	$B_{old(1)}$	$B_{old(2)}$	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	0	1	0	0	0	0	0	0	OFF
Medium lactose	0	1	1	1	0	0	1	0	ON

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

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

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

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

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” ($.135 \text{ min}^{-1}$), 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:

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

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

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 $\tau_B = 2 \text{ min}$) of mRNA on the production of β -galactosidase.
- A_1 to model the delayed action of A on the production of mRNA by $\tau_M = .1 \text{ min}$.

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

- M_{old} since $\widetilde{h}_M = 1.5$ is approximately 1 time-step.
- A_{old} since $\widetilde{h}_A = 1.26$ 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., $B_{\text{old}(1)}, B_{\text{old}(2)}, \dots, B_{\text{old}(22)}$ 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

$$f_M = A_1 \vee (M \wedge \overline{M_{old}})$$

$$f_{M_1} = M$$

$$f_{M_2} = M_1$$

$$f_{M_{old}} = \overline{A_1} \wedge M$$

$$f_A = (B \wedge L_m) \vee L \vee (A \wedge \overline{A_{old}} \wedge \overline{B})$$

$$f_{A_1} = A$$

$$f_{A_{old}} = ((\overline{B} \vee \overline{L_m}) \wedge \overline{L}) \wedge A$$

$$f_B = M_2 \vee (B \wedge \overline{B_{old(2)}})$$

$$f_{B_{old(1)}} = \overline{M_2} \wedge B$$

$$f_{B_{old(2)}} = \overline{M_2} \wedge B_{old(1)}$$

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_{old}	B	$B_{old(1)}$	$B_{old(2)}$	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	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.

$$\frac{dM}{dt} = \alpha_M \frac{1 + K_1(e^{-\mu\tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu\tau_M} A_{\tau_M})^n} + \Gamma_0 - \tilde{\gamma}_M M$$

$$\frac{dB}{dt} = \alpha_B e^{-\mu\tau_B} M_{\tau_B} - \tilde{\gamma}_B B$$

$$\frac{dA}{dt} = \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \tilde{\gamma}_A A$$

$$\frac{dP}{dt} = \alpha_P e^{-\mu(\tau_B + \tau_P)} M_{\tau_B + \tau_P} - \tilde{\gamma}_P P$$

$$\frac{dL}{dt} = \alpha_L P \frac{L_e}{K_{L_e} + L_e} - \beta_{L_e} P \frac{L}{K_{L_e} + L} - \alpha_A B \frac{L}{K_L + L} - \tilde{\gamma}_L L$$

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

$$\mu = .03 \text{ min}^{-1}, \quad \tilde{\gamma}_A = \gamma + \mu = .044, \quad \tilde{\gamma}_B = \gamma + \mu = .031, \quad \tilde{\gamma}_M = \gamma + \mu = .441.$$

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

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 ≈ 12 min.
- Ignore (all $\ll 12$): $\tau_M = .10$ min, $\tau_B = 2$ min, $\tilde{h}_M = 1.572$ min.
- Introduce **dilution & degradation variables**: $A_{old}, B_{old}, L_{old}, P_{old}$.

Proposed model

$$f_M = A \vee (M \wedge \overline{M_{old}})$$

$$f_{M_{old}} = \overline{A} \wedge M$$

$$f_A = (B \wedge L) \vee (L \wedge L_e) \vee (A \wedge \overline{A_{old}} \wedge \overline{B})$$

$$f_{A_{old}} = (\overline{B} \vee \overline{L}) \wedge (\overline{L} \vee \overline{L_e}) \wedge A$$

$$f_L = ((P \wedge L_{em}) \vee L_e) \vee ((L \wedge \overline{L_{old}}) \wedge (\overline{B} \wedge \overline{P}))$$

$$f_B = M \vee (B \wedge \overline{B_{old}})$$

$$f_{B_{old}} = \overline{M} \wedge B$$

$$f_P = M \vee (P \wedge \overline{P_{old}})$$

$$f_{P_{old}} = \overline{M} \wedge P$$

$$f_{L_{old}} = ((\overline{P} \vee \overline{L_{em}}) \wedge \overline{L_e}) \wedge L$$

A Boolean model incorporating dilution and degradation

Justification for f_A

$$f_A = (B \wedge L) \vee (L \wedge L_e) \vee (A \wedge \overline{A_{\text{old}}} \wedge \overline{B})$$

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 lactose 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 & galactose.

Justification for f_L

$$f_L = ((P \wedge L_{em}) \vee L_e) \vee ((L \wedge \overline{L_{\text{old}}}) \wedge (\overline{B} \wedge \overline{P}))$$

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 β -galactosidase).

A Boolean model incorporating dilution and degradation

Model:

$$f_M = A \vee (M \wedge \overline{M_{\text{old}}})$$

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

$$f_A = (B \wedge L) \vee (L \wedge L_e) \vee (A \wedge \overline{A_{\text{old}}} \wedge \overline{B})$$

$$f_{A_{\text{old}}} = (\overline{B} \vee \overline{L}) \wedge (\overline{L} \vee \overline{L_e}) \wedge A$$

$$f_L = ((P \wedge L_{em}) \vee L_e) \vee ((L \wedge \overline{L_{\text{old}}}) \wedge (\overline{B} \wedge \overline{P}))$$

$$f_B = M \vee (B \wedge \overline{B_{\text{old}}})$$

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

$$f_P = M \vee (P \wedge \overline{P_{\text{old}}})$$

$$f_{P_{\text{old}}} = \overline{M} \wedge P$$

$$f_{L_{\text{old}}} = ((\overline{P} \vee \overline{L_{em}}) \wedge \overline{L_e}) \wedge L$$

Fixed points:

Ext. Lactose	L_e	L_{em}	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	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