Synthetic Biology Assignment

Zheng-Xiang Ye

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Problem 1. Write down the equation in Box 1. What are (1) u, (2) v, (3) α_1 and (4) α_2?

Box 1 Equation:

$$\frac{du}{dt} = \frac{\alpha_1}{1+v^\beta} - u \tag{1}$$

$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^\gamma} - v \tag{2}$$

(1) u is the concentration of repressor 1.
(2) v is the concentration of repressor 2.
(3) α_1 is the effective rate of synthesis of repressor 1.
(4) α_2 is the effective rate of synthesis of repressor 2.

Problem 2. How did they change the rates of synthesis of the repressors?

The rates of synthesis of the repressors (α_1 and α_2) are modified by exchanging the downstream ribosome binding sites. The promoter sequences remain the same.

Problem 3. Read the Supplementary Information. What are A, B, C... ? How did they turn pIKE plasmid (repressor pairs) into a bistable circuit?

A to G are different sequences of ribosomal binding sites. The sequence A has the highest translational efficiency. The efficiency decreases in alphabetic order and G has the lowest efficiency.

To make a bistable circuit, the rates of synthesis of the repressors must be balanced. The bistability of a circuit can be identified by plotting the nullclines ($\frac{du}{dt} = 0$, $\frac{dv}{dt} = 0$) and counting the number of intersections. Figures 2a and 2b (in the paper) clearly illustrate the distinctions between monostable and bistable circuits. A bistable circuit has three intersections in the figure, whereas a monostable circuit only has one.

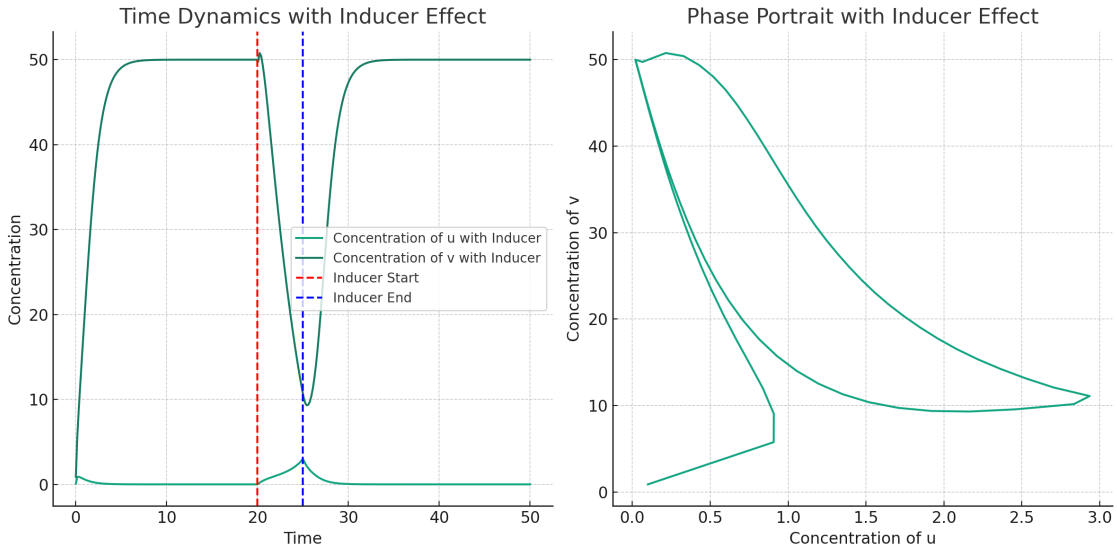
Therefore, the translational efficiency of sequence A is too strong so that it falls out of the range that creates three intersections in the nullcline plot. On the other hand, B to G have moderate efficiencies that can maintain bistable circuits.

Problem 4. Read the Methods. "Because JM2.300 contains no λ repressor and carries a nonfunctional Lac repressor (lacI22), it is an ideal host for the toggle switch." Why is this important for a synthetic circuit?

The authors use λ and Lac repressors in their experimental settings. If they use a strain containing intrinsic expressions of these repressors, the result will be influenced by these intrinsic expressions and deviate from their model. In addition, this entails extra efforts to measure the intrinsic expressions if they still proceeded with such strain. Generally, having less variables in experiments often produces more reliable results and reduces the variation of experimental measurements. Thus, a strain such as JM2.300 should be favored if λ and Lac repressors are the elements in the circuit.

Problem 5 (Optional). Refer to our lecture slides (p. 40-41). Ask ChatGPT to write a set of Python code to plot the following figures.

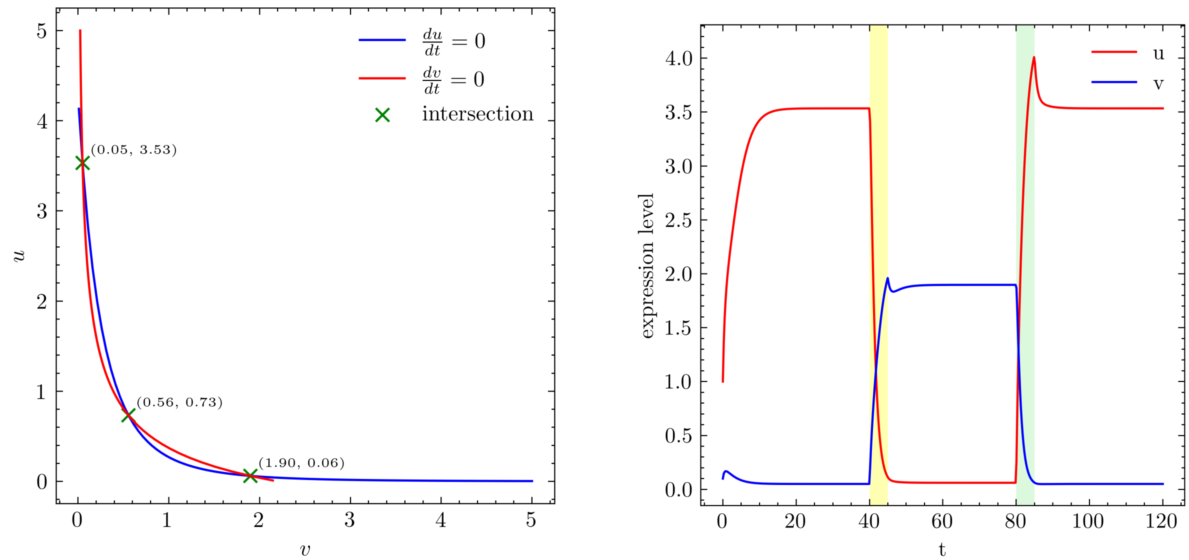
Note:
4/11: I would like to try solving this problem myself first and see if I can reproduce the figures.
4/18: After solving this problem myself, I tried using ChatGPT 4 to see if it is capable of understanding the question and analyzing the mathematical equations. It turned out that ChatGPT 4 performed better than I expected. It understood the equations correctly and produced figures that represent the behavior of monostable circuits (see the figure below). However, it failed to find the parameter space that exhibit bistability even after a parameter sweep (the generated code used for parameter sweeping looked correct but perhaps it searched the wrong range of parameters).
Link for the chat history



As mentioned in the answer to problem 3, the nullcline plots can be utilized to explore the parameters suitable for creating a bistable circuit.

As an example, Figure 1 shows the result of a set of parameters ($\alpha_1 = 4$, $\alpha_2 = 2.2$, $\beta = 4$, $\gamma = 2.5$) that exhibits bistability. Note that the steady state expression levels in Figure 1b correspond to the coordinates of the two intersections ($v = 0.05, u = 3.53$) and ($v = 1.90, u = 0.06$). On the other hand, creating a monostable circuit is easier. Figure 2 shows two examples of the monostable circuits with $\alpha_1 > \alpha_2$ and $\alpha_1 < \alpha_2$, respectively.

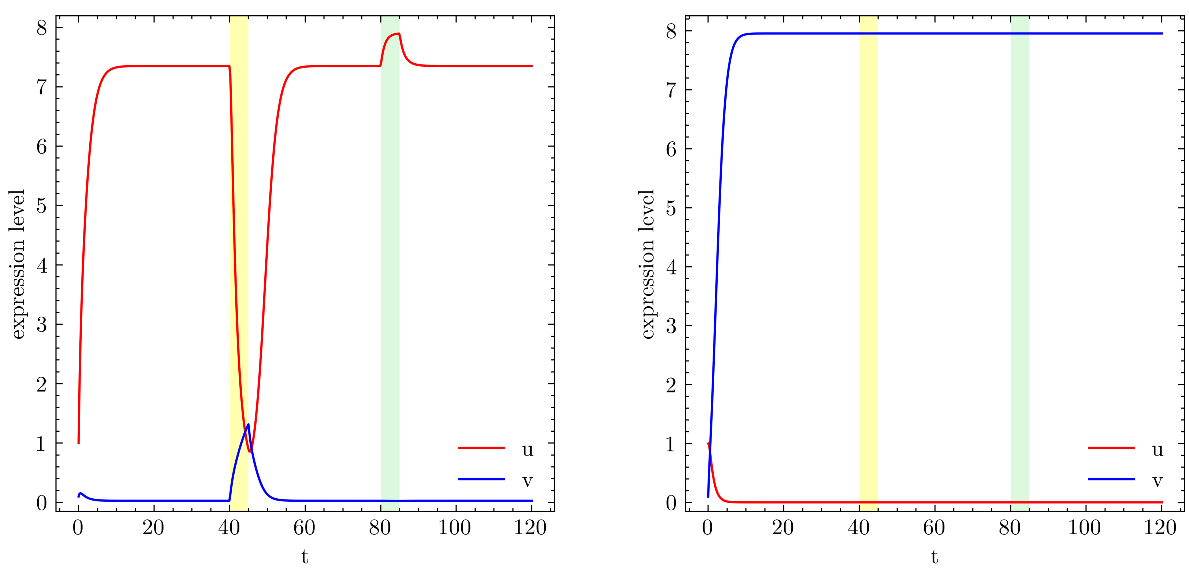
Code for producing Figure 1 and Figure 2



(a) Nullcline plot

(b) Switching between two steady states

Figure 1: Example of a bistable circuit. The parameters are set to ($\alpha_1 = 4$, $\alpha_2 = 2.2$, $\beta = 4$, $\gamma = 2.5$). The yellow and green regions in (b) indicate the time the inducer for v (yellow) and u (green) are added, respectively. The net effect of adding an inducer can be modeled by changing the cooperativity parameter γ or β. Specifically, γ and β are temporarily set to 0.5 in the yellow and green regions, respectively.



(a) Monostable circuit ($\alpha_1 > \alpha_2$)

(b) Monostable circuit ($\alpha_1 < \alpha_2$)

Figure 2: Example of two monostable circuits. (a) $\alpha_1 = 8$, $\alpha_2 = 2$, $\beta = 3$, $\gamma = 2$. (b) $\alpha_1 = 2$, $\alpha_2 = 8$, $\beta = 3$, $\gamma = 2$. The yellow and green regions share the same conditions with those in Figure 1b.