

CALIFORNIA INSTITUTE OF TECHNOLOGY
Biology and Biological Engineering (BBE)

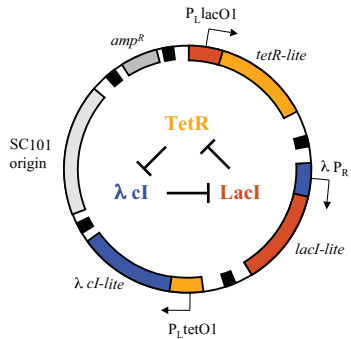
BE 150

M. Elowitz and R. M. Murray
Spring 2014

Problem Set #4

Issued: 24 April 2014
Due: May 1 2014

1. (Repressilator modeling and robustness) The repressilator is a synthetic genetic transcriptional circuit that is design to produce a sustained oscillation in cells. A schematic diagram and simple differential equation model are shown below:



$$\frac{dm_1}{dt} = \frac{\alpha}{1 + \left(\frac{p_3}{K_m}\right)^n} - \delta m_1$$

$$\frac{dp_1}{dt} = \beta m_1 - \gamma p_1$$

$$\frac{dm_2}{dt} = \frac{\alpha}{1 + \left(\frac{p_1}{K_m}\right)^n} - \delta m_2$$

$$\frac{dp_2}{dt} = \beta m_2 - \gamma p_2$$

$$\frac{dm_3}{dt} = \frac{\alpha}{1 + \left(\frac{p_2}{K_m}\right)^n} - \delta m_3$$

$$\frac{dp_3}{dt} = \beta m_3 - \gamma p_3$$

- (a) Build a SimBiology model of the repressilator that captures the mRNA and protein dynamics shown to the right and simulate the system using the following parameters: $\alpha = 0.5$, $n = 2$, $K_m = 40$, $\delta = 0.0058$, $\gamma = 0.0012$, $\beta = 0.116$. (Note: you should use the “unknown” kinetic law in SimBiology to implement these dynamics.) Show that some (reasonable) initial conditions generate sustained oscillations while other initial conditions do not.
- (b) Suppose the protein half-life suddenly decreases by half. Which parameter(s) will change and how? Simulate what happens. What if the protein half-life is doubled? How do these two changes affect the oscillatory behavior?
- (c) Now assume that there is leakiness in the transcription process. How does the system’s ODE change? Simulate the system with a small leakiness (say, 5×10^{-3}) and comment on how it affects the oscillatory behavior.
2. (Autoinhibition with transcriptional delay; based on Lewis, J., 200x, DOI 10.1016/S0960-9822(03)00534-7) Consider the following delayed differential equations:

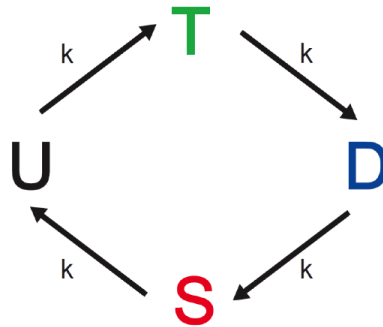
$$\frac{dm(t)}{dt} = f(p(t - T_m)) - \delta m(t) \quad \frac{dp(t)}{dt} = \beta m(t - T_p) - \gamma p(t)$$

with

$$f(p) = \frac{k}{1 + p^2/p_0^2}$$

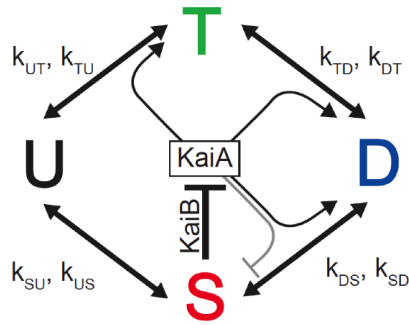
The function $f(p)$ represents the action of the inhibitory protein as a dimer and the variables m and p are mRNA and protein concentrations. The parameter T_m is the delay from initiation of transcription and the arrival of mature mRNA into the cytoplasm, T_p is the delay from initiation of translation to the emergence of a complete functional protein. The parameters δ and γ are degradation of mRNA and protein.

- (a) Implement the delayed differential equation model above in MATLAB. Specifically, write a function which takes in the current and delayed protein and mRNA levels (assume these are known) as input and returns the vector $[dm/dt; dp/dt]$. Choose parameters from the paper to obtain sustained oscillations and plot the mRNA and protein concentrations. You can implement your own numerical integrator or use `dde.m` from the course website, which takes a function as one of its arguments.
- (b) Comment on the robustness of sustained oscillations with respect to reduction in protein synthesis rate.
- (c) Leave parameters as in a) and change only one parameter to obtain damped oscillations and plot mRNA and protein concentrations. Do not change the protein synthesis rate. Comment on how the change of parameter might affect damping of the oscillation.
3. (Limit cycle behaviors in simple chemical reactions) The KaiABC circuit is a protein-based circadian oscillator found in cyanobacteria. The main protein, KaiC, has two distinct phosphorylation sites. Therefore it has four possible states - unphosphorylated (U), phosphorylated at the threonine site (T), phosphorylated at the serine site (S), or phosphorylated at both sites (D).
- (a) Consider the following KaiC reaction scheme discussed during the circadian clock lecture. At first glance, a system like this appears like it might be able to support self-sustaining oscillations:



- i. Write down a three-dimensional system of differential equations describing this system. Remember that the total amount of KaiC, $C_{tot} = T + U + D + S$, is constant. Assume that $C_{tot} = 3.4\mu M$ and $k = 0.116 h^{-1}$.
- ii. Solve for the fixed point(s).
- iii. Determine the stability of the fixed point using linear stability analysis. Find the eigenvalues of the Jacobian of your ODE system. A fixed point is stable if and only if all eigenvalues λ_i have $\text{Re}(\lambda_i) \leq 0$. Is the fixed point stable?
- iv. Simulate the dynamics of the system from three different initial conditions, running the simulations for 200 hrs. Plot the resulting trajectories in 3D phase space using the Matlab command `plot3`.
- v. Simulate the total phosphorylation of KaiC ($T + D + S$) versus time using `ode45` for each of the three initial conditions, and plot. Can you explain intuitively why the dynamics have this form, and why this scheme does not appear to generate self-sustaining oscillations? Would you expect the system to behave differently if there were only one molecule of KaiC?

(b) Cyclic scheme with feedback/nonlinearity: Now consider the same system with feedback:



$$U = C_{tot} - S - T - D$$

$$k_{XY} = k_{XY}^{basal} + \frac{k_{XY}^A * A_{act}}{A_{act} + K_{1/2}}$$

$$A_{act} = \max\{0, A_{tot} - 2S\}$$

where k_{XY} is the rate constant (which depends indirectly on S) for the conversion from phosphoform X to phosphoform Y, and the constants C_{tot} , A_{act} , k_{XY}^{basal} , k_{XY}^A , and $K_{1/2}$ are given in the following table.

Category	Process	Parameter name	Value
Basal rates (- KaiA)	U → T		0 h ⁻¹
	T → D		0 h ⁻¹
	S → D		0 h ⁻¹
	U → S		0 h ⁻¹
	T → U		0.21 h ⁻¹
	D → T		0 h ⁻¹
	D → S		0.31 h ⁻¹
	S → U		0.11 h ⁻¹
Maximal Effect of KaiA	U → T		0.479077 h ⁻¹
	T → D		0.212923 h ⁻¹
	S → D		0.505692 h ⁻¹
	U → S		0.0532308 h ⁻¹
	T → U		0.0798462 h ⁻¹
	D → T		0.1730000 h ⁻¹
	D → S		-0.319385 h ⁻¹
	S → U		-0.133077 h ⁻¹
Other	Concentration of KaiA causing half-maximal effect on KaiC	$K_{1/2}$	0.43 μM
	Total concentration of KaiA	A_{tot}	1.3 μM
	Total concentration of KaiC	C_{tot}	3.4 μM

- Use *ode45* to simulate this system in (T, D, S) phase space for three different initial conditions of your choosing, plotting the results in 3D phase space with *plot3*. Make sure that your initial conditions T_0, D_0, S_0 are with respect to the value of C_{tot} , i.e., $T_0 + D_0 + S_0 \leq C_{tot}$. Run the simulations for 200 h.
- Plot total phosphorylation of KaiC (T + D + S) versus time for each of the three initial conditions.
- What is the period T of the oscillator? Does it depend on the initial conditions? Why or why not?
- Why do the initial (transient) dynamics differ depending on initial conditions?

- (c) Phase response curve: A key characteristic of circadian clocks is their so-called phase response curve (PRC), which describes how the phase of the clock changes in response to a stimulus/perturbation such as exposure to light for a defined duration. A PRC is a plot of the change in phase of the clock (y axis) versus the phase (subjective time) at which the perturbation is applied (x axis). The phase change is evaluated by comparing the phase of the perturbed clock to the phase expected in the absence of perturbation several periods after the perturbation to allow the phase of the perturbed clock to stabilize.
- i. For the simple system examined above, apply a perturbation described by the vector $\delta = (\delta T, \delta D, \delta S) = (-0.05\mu M, -0.05\mu M, -0.05\mu M)$ at each hour of the day, that is for 24 different times evenly spaced within $[103.5 \text{ h}, 103.5 \text{ hours} + T)$, where T is the period you determined in 3b(iii). (Note that a given system is perturbed only once; you need to run 24 different simulations.) Calculate the trajectories of each of the perturbed systems for at least six periods following the perturbation. Also, calculate the trajectory of an unperturbed clock for at least seven periods following 103.5 hours. Plot the total phosphorylation for the 1st, 6th, 12th, and 18th perturbations versus time, for $t = [100, 200]$. On the same plot, show the total phosphorylation of the unperturbed clock over the same time period.
 - ii. Now calculate and plot the phase response curve for those 24 perturbations. Define the time at which the perturbation is applied as $24 * (t_{\text{perturb}} - t_{\text{precedingpeak}})/T$. t_{perturb} is the time at which the perturbation is applied, $t_{\text{precedingpeak}}$ is the time of the last peak of total phosphorylation immediately preceding t_{perturb} , and T is the period of the oscillator. The time should lie in the interval $[0 \text{ h}, 24 \text{ h}]$. (This formula normalizes time to that of an idealized 24-hour clock, resulting in a measure called circadian time [CT], which is standard in the field.) Define the phase change after perturbation, measured in the CT shift of the total phosphorylation peak, such that it lies in the interval $[-12 \text{ h}, +12 \text{ h}]$. To determine the phase change, consider the phase of the perturbed oscillator beginning $5 * T$ after perturbation compared to that of the unperturbed oscillator. Match the trajectories plotted in 3c(i) to their circadian times.
 - iii. Describe the implications of this PRC for you if you were a cyanobacterium traveling from Los Angeles to Beijing via a Star Trek-style (instantaneous) transporter. The time in Beijing is 15 hours ahead of the time in Los Angeles; in the date-agnostic PRC in 3c(ii), in which phase changes lie in $[-12 \text{ h}, 12 \text{ h}]$, this time difference would be equivalent to a 9-hour phase delay (-9 h). If you could perturb your clock with perturbation δ once per day, and the phase change were instantaneous (for the sake of simplicity), describe a scheme for synchronizing your circadian clock with Beijing time?

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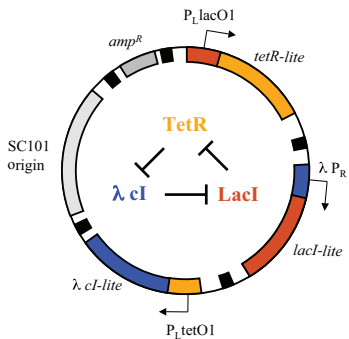
Bi 250b

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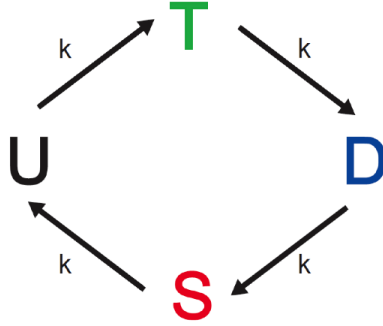
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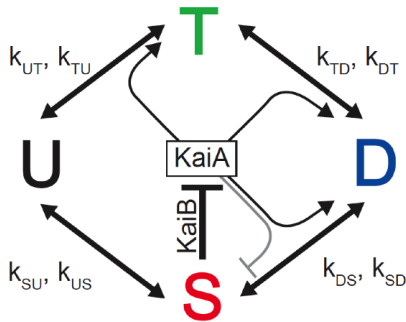
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