NON LINEAR DYNAMICS PROJECT REPORT

Analysis of dynamics of regulatory and signaling pathways in the cell

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Abstract—The physiological responses of cells to external and internal stimuli are governed by genes and proteins interacting in complex networks whose dynamical properties are impossible to understand by intuitive reasoning alone. To build models to understand how these systems work, one must develop a precise mathematical description of molecular circuitry and some intuition about the dynamical properties of regulatory networks. In this review, we have shown how to create mathematical representations (non-linear differential equations) of some simple signalresponse elements, and how certain feedback and feedforward signals can create diverse types of responses: sigmoidal switches (buzzers), transient responses (sniffers), hysteretic switches (toggles), and oscillators (blinkers). [1]

I. Linear and hyperbolic signal-response curves

We begin our study of regulatory and signalling pathways in cells with two simple examples of protein dynamics whose rate equations are given below -
(a) $dR/dt = k_o + k_1 S - k_2 R$ ..... Synthesis and Degradation
(b) $dR_P/dt = k_1 S(R_T - R_P) - k_2 R_P$ .... Phosphorylation and Dephosphorylation
In case (a), S is signal strength (e.g. concentration of mRNA) and R is response magnitude (e.g. concentration of protein).

In case (b), R_P is the phosphorylated form of the response element (which we suppose to be the active form), R_P = [RP], and R_T = R + R_P = total concentration of the response element. A steady-state solution of a differential equation, $dR/dt = f(R)$, is a constant, R_{ss}, that satisfies the algebraic equation $f(R_{ss}) = 0$. Solving for R_{ss} in the two cases gives the following -

$$(a) R_{ss} = \frac{k_o + k_1 S}{k_2} \qquad (b) R_{P,ss} = \frac{R_T S}{(k_2/k_1) + S}$$

These relations lead to hyperbolic and linear signal response curves shown in Fig 1 and 2 respectively.

II. Sigmoid signal-response curves

In some cases, the phosphorylation and dephosphorylation reactions may be governed by Michaelis-Menten kinetics-

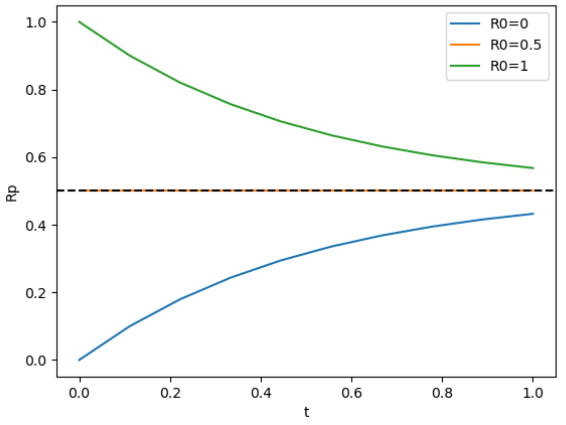
$$\frac{dR_P}{dt} = \frac{k_1 S(R_T - R_P)}{k_{m1} + R_T - RP} - \frac{k_2 R_P}{k_{m2} + R_P}$$

In this case, the steady-state concentration of the phosphorylated form is a solution of a quadratic equation and the biophysically acceptable solution ($0 < R_P < R_T$) is given as follows-

$$\frac{R_{P,ss}}{R_T} = G(k_1, k_2 S, \frac{K_{m1}}{R_T}, \frac{K_{m2}}{R_T})$$

where the 'Goldbeter-Koshland' function, G, is defined as:

$$G(u, v, J, K) =$$



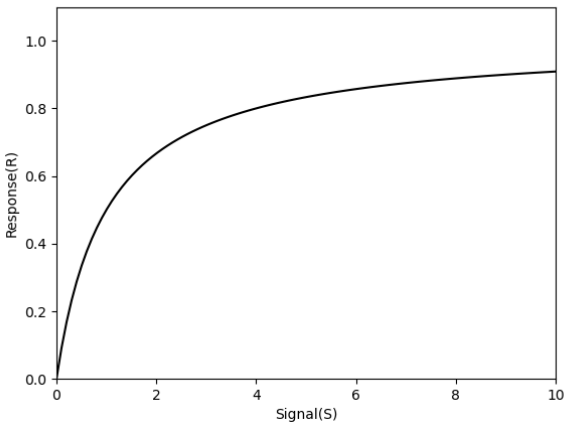


Fig. 1: Solution and Signal response curve for Hyperbolic Response

$$\frac{2uK}{v - u + vJ + uK + \sqrt{(v - u + vJ + uK)^2 - 4(v - u)uK}}$$

The plot of $R_{P,ss}$ versus S comes out as a sigmoidal curve if J and K are both $<< 1$ (shown in Fig 3). This mechanism for creating a switch-like signal response curve is called *zero-*

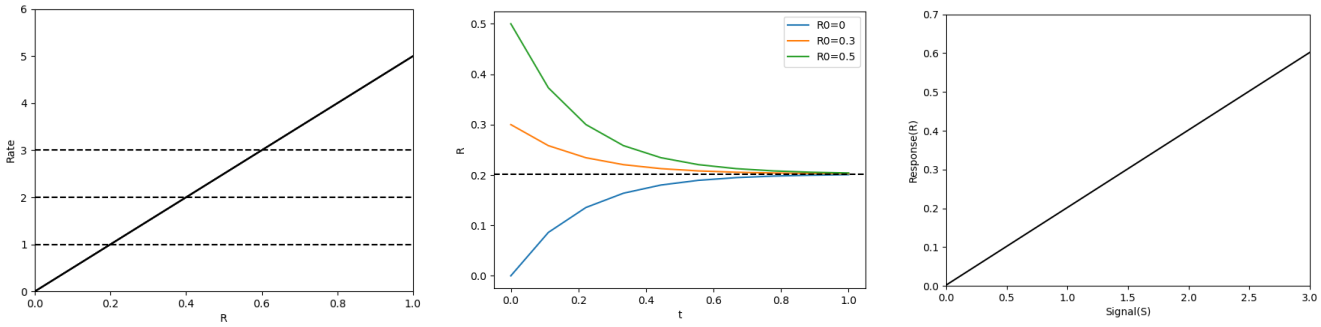


Fig. 2: Rate, Solution and Signal response curve for Linear response

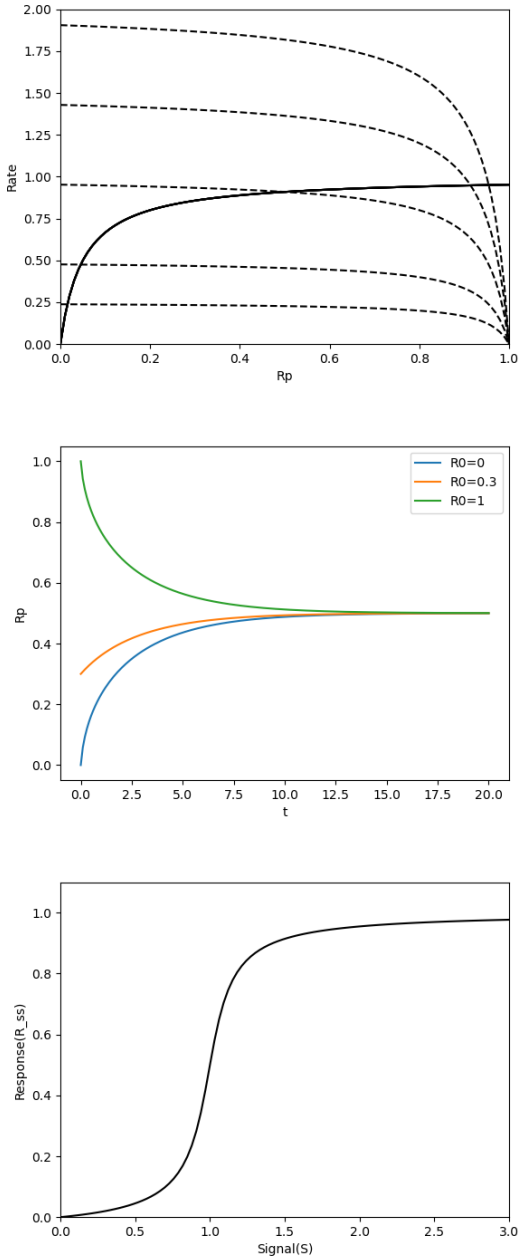


Fig. 3: Rate, Solution and Signal responce curve of sigmoid response

order ultrasensitivity. Moreover, the relationship is graded, i.e., the response increases continuously with signal strength.

It is also reversible in the sense that if the signal strength is changed from $S_{initial}$ to S_{final}, the response at S_{final} is the same whether the signal is being increased or decreased.
Like a **buzzer** or a laser pointer, to activate the response one must push hard enough on the button, and to sustain the response one must keep pushing. When one lets up on the button, the response switches off at precisely the same signal strength at which it switched on.

III. Perfectly Adapted Signal-Response Curves

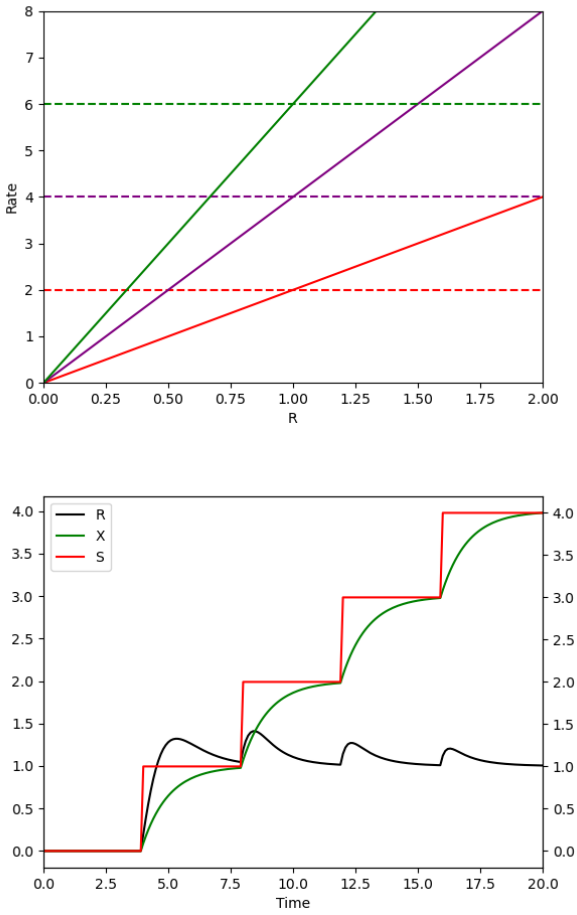


Fig. 4: Rate and Solution curve

By supplementing the simple linear response element (as in the first case) with a second signaling pathway through another species X, we can create a response mechanism that

exhibits perfect adaptation to the signal. Perfect adaptation means that although the signaling pathway exhibits a transient response to changes in signal strength, its steady-state response R_{ss} is independent of S (see Fig 4). Such behaviour is typical of chemotactic systems, which respond to an abrupt change in attractants or repellents, but then adapt to a constant level of the signal. Our own sense of smell operates this way, so we refer to this type of response as a **'sniffer'**.

IV. Positive feedback: irreversible switches

In some cases, the signal influences response via two parallel pathways (through another entity X) which work in opposite directions. Alternatively, some component of a response pathway may feed back on the signal. Signal can be positive, negative or mixed.

Positive feedback can be of two kinds. Consider the following two cases-

- R (response) activates protein E (by phosphorylation) and EP enhances the synthesis of R - (**mutual activation**)
- R inhibits E and E promotes the degradation of R - (**antagonism**)

In either case, positive feedback may create a discontinuous switch, meaning that the cellular response changes abruptly and irreversibly as signal magnitude crosses a critical value.

For example, in the first case (Fig 5), as the signal strength increases, the response increases but is low and jumps to a high value at a certain signal strength S_{crit}. If the signal strength is now reduced, it decreases slowly while still remaining on the upper branch (i.e., the switch is **irreversible**, unlike the sigmoidal response). In the region $0 < S < S_{crit}$, the system is bistable, i.e., has two stable state (one with high response and other with low response), separated by an unstable steady state (on the intermediate branch - the dashed line).
In the second case (Fig 6), once the switch jumps to the on state and signal strength starts reducing, at (another) critical value the switch goes back to the off state. For intermediate stimulus strengths ($S_{crit1} < S < Scrit2$), the response of the system can be either small or large, depending on how S was changed. This sort of two-way, discontinuous switch is often referred to as **hysteresis**.

The two kinds of discontinuous responses described above are also referred to as the **one-way switch** and the **toggle switch** for obvious reasons.

The signal-response curves in the above two cases can be called 'one-parameter bifurcation diagrams'. The parameter is signal strength (manipulatable by the experimenter). The steady-state response, on the Y axis, is an indicator of the behaviour of the control system as a function of the signal. At S_{crit}, the behaviour of the control system changes abruptly and irreversibly from low response to high response (or vice versa). Such points of qualitative change in the behaviour of a nonlinear control system are called bifurcation points, in this case, a 'saddle-node bifurcation point'.

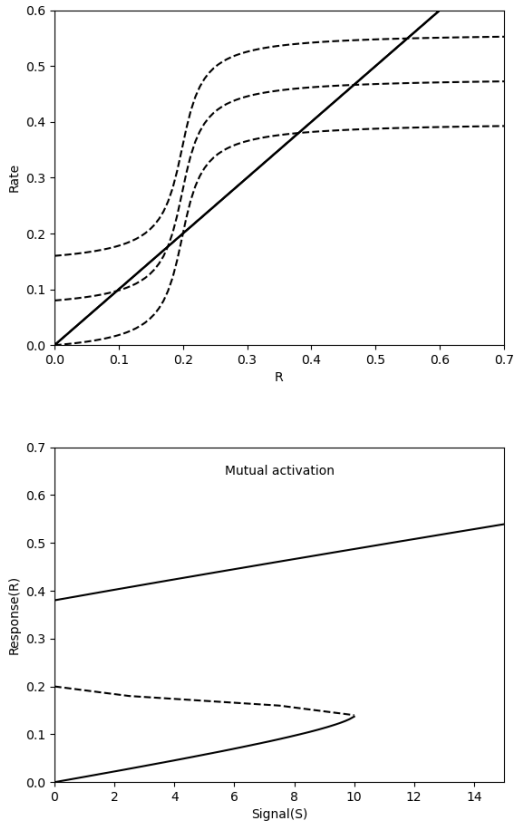


Fig. 5: Mutual Activation

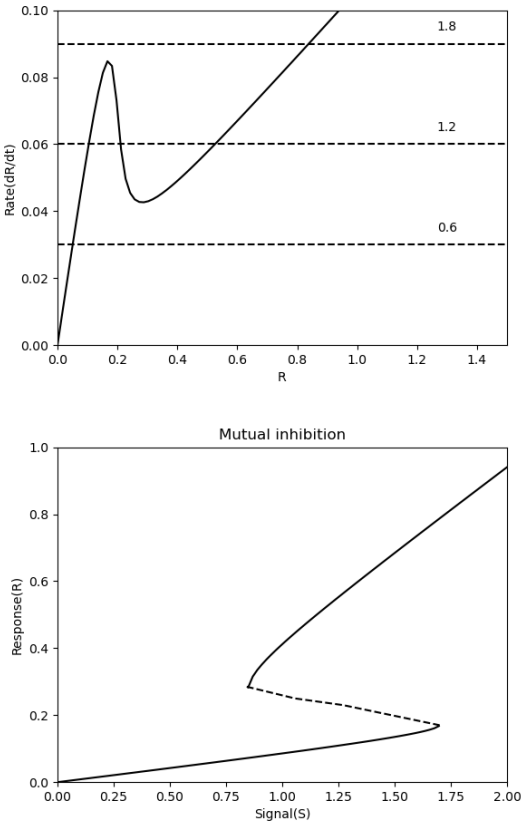


Fig. 6: Mutual inhibition

V. NEGATIVE FEEDBACK: HOMEOSTASIS AND OSCILLATIONS

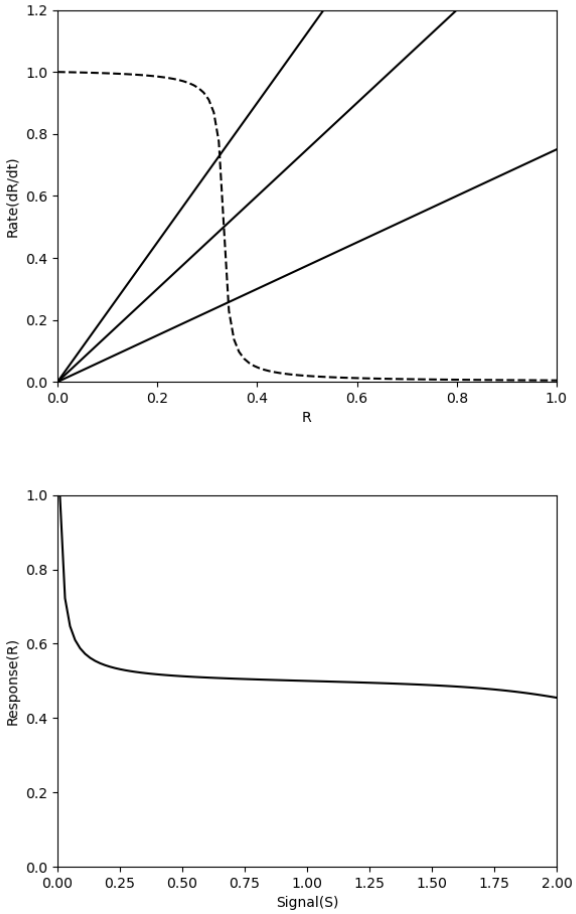


Fig. 7: Rate and Signal response curve of Negative Feedback: Homeostasis[2]

- **Homeostasis**:-In negative feedback, the response counteracts the effect of the stimulus. The response element, R, inhibits the enzyme catalysing its synthesis; hence, the rate of production of R is a sigmoidal decreasing function of R (Fig 7). The signal S is the demand for R for consumption. The steady state concentration of R is confined to a narrow window for a broad range of S, because the supply of R adjusts to its demand. This type of regulation, is called homeostasis. It differs from a sniffer because stepwise increases in S do not generate transient changes in R.

$$\frac{dR}{dt} = k_o E(R) - k_2 S.R$$
$$E(R) = G(k_3, k_4 R, J_3, J_4)$$

- **Oscillations**:- Negative feedback can also create an oscillatory response.But with two components (R,X) the system will exhibit damped oscillations to a stable steady state. For sustained oscillations, we need atleast three components where the third component (Y) introduces a time delay in the feedback loop, causing the control system repeatedly to overshoot and undershoot its steady state.

$$\frac{dX}{dt} = k_o + k_1 S - k_2 X + k_2' R_P.X$$
$$\frac{dY_P}{dt} = \frac{k_3 X(Y_T - Y_P)}{K_{m3} + Y_T - Y_P} - \frac{k_4 Y_P}{K_{m4} + Y_P}$$
$$\frac{dR_P}{dt} = \frac{k_5 Y_P(R_T - R_P)}{K_{m5} + R_T - R_P} - \frac{k_6 R_P}{K_{m6} + R_P}$$

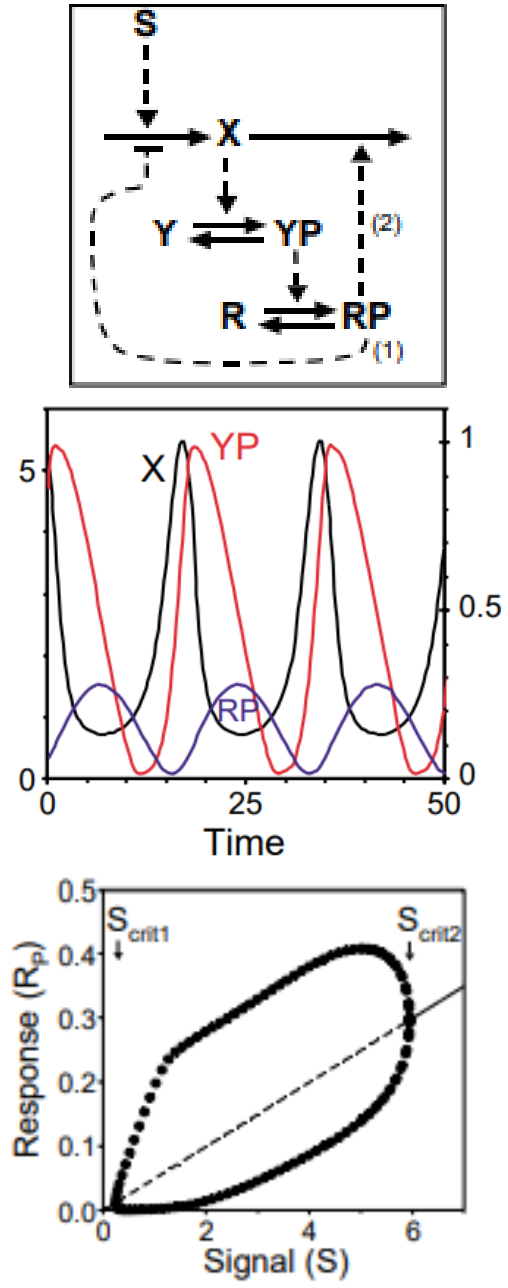


Fig. 10: Wire Diagram Solution, Signal response curve of Negative Feedback

As shown in Fig 8 above, the steady state response is unstable for $S_{crit1} < S < S_{crit2}$. Within this range, $R_{p(t)}$ oscillates between R_{Pmin} and R_{Pmax}. Here, $S_{crit1} and S_{crit2}$ are bifurcation points, where the steady-state response changes its stability and therefore a 'Hopf bifurcation' occurs.As S moves away from either bifurcation point, the amplitude of oscillation increases.

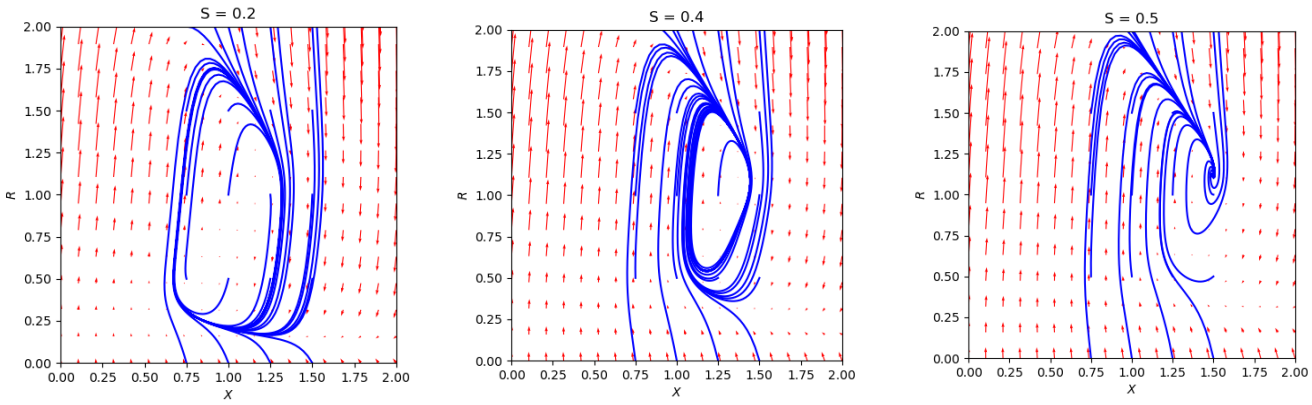


Fig. 8: Activator-inhibitor oscillators

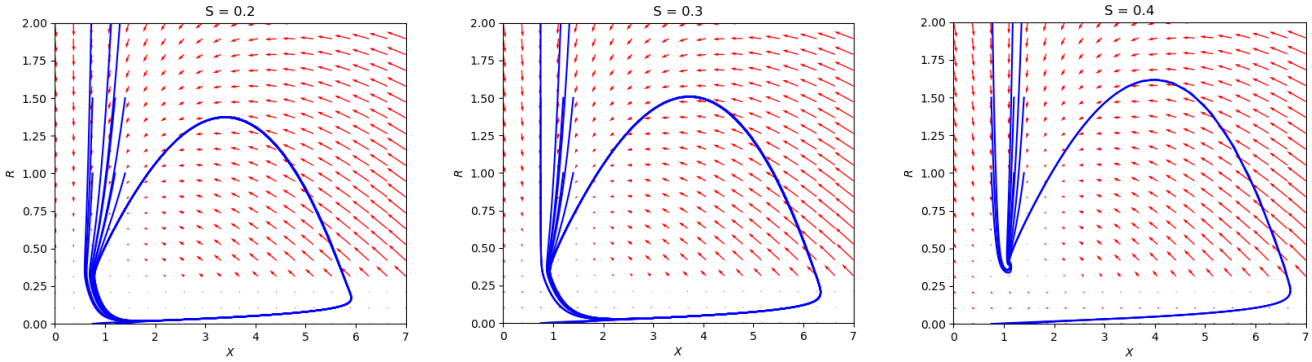


Fig. 9: Substrate-depletion oscillators [3]

VI. Positive and Negative Feedback: Oscillators

For Oscillations we often need both positive and negative feedback. The positive feedback loop creates a bistable system (a toggle switch) and the negative-feedback loop drives the system back and forth between the two stable steady states. Oscillators of this sort have two possibilities.

a. Activator-inhibitor oscillators

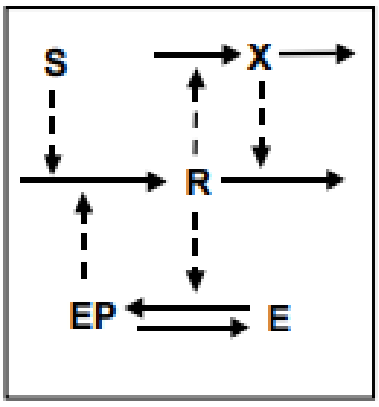


Fig. 11: Wire Diagram of Activator-inhibitor oscillators

R is created in an autocatalytic process, and then it promotes the production of an inhibitor X, which speeds up R removal. First, R builds up, then comes X to curb R back down, then X disappears and R can rise again.

$$\begin{aligned}\frac{dR}{dt} &= k_o E_P(R) + k_1 S - k_2 R - K_2^{'}.R \\ \frac{dX}{dt} &= k_5 R - k_6 X \\ E(R) &= G(k_3, k_4 R, J_3, J_4)\end{aligned}$$

As a result, the variables, R(t) and X(t), oscillate around the steady state on a closed orbit (see Fig 9) called a stable limit cycle. Such behaviour is called a hysteresis oscillator, and the closed orbit is called a hysteresis loop. The classic example of an activator-inhibitor system is cyclic AMP production.

b. Substrate-depletion oscillators

$$\begin{aligned}\frac{dX}{dt} &= k_1 S - [k_o^{'} + k_o E_P(R)]X \\ \frac{dR}{dt} &= k_o E_P(R)].X - k_2 R \\ E_P(R) &= G(k_3 R, J_4\end{aligned}$$

X is converted into R in an autocatalytic process. Suppose, initially, X is abundant and R is scarce. As R builds up, the production of R accelerates until there is an explosive conversion of the entire pool of X into R. Then the autocatalytic reaction shuts off for lack of substrate, X. R is degraded, and X must build up again before another burst of R is produced.

Bifurcations occurring at substrate-depletion oscillator are different than bifurcations at negative-feedback oscillator. In

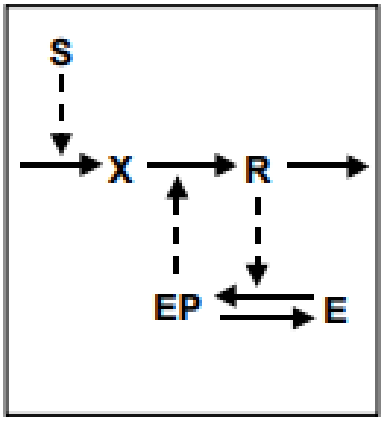


Fig. 12: Wire Diagram of Substrate-depletion oscillators

negative-feedback case they were 'supercritical Hopf bifurcation' and in substrate-depletion case they were 'subcritical Hopf bifurcations'.

In both cases as we vary S we pass a Hopf bifurcation at the two points - $S_{crit1} and S_{crit2}$. In the previous case, it is a supercritical Hopf bifurcation, and the oscillatory solutions appear, at first, with small amplitude, perhaps too small to generate a useful response. On the other hand, in this case, as S passes the subcritical Hopf bifurcation, stable oscillations of large amplitude appear abruptly. The control system immediately generates a large and robust response. When S is being changed in the opposite direction, the large amplitude periodic solutions disappear just as abruptly. Hence, subcritical Hopf bifurcations provide a general mechanism for hysteretic transitions between a stable steady state and a stable, large amplitude oscillation. In biophysical control systems, where membrane potential oscillations can be measured with great accuracy, it is easy to distinguish the difference between sub- and supercritical Hopf bifurcations.

VII. Conclusion

All the mechanisms discussed here including sniffers, buzzers, toggles, blinkers, switches and oscillators are an important part of all biological processes that occur inside any living thing. The understanding of these structures and even more importantly building a mathematical model of them with which we have described extremely complex processes using non-linear equations will be revolutionary in multiple biological fields.

Stimuli and response contribute to the very definition of life. By simulating them we are able to predict the behaviour of a cell and even of an organism. This also demonstrates the powerful ability of simple chemical reactions to be able to give us an extremely wide variety of dynamics. Inspiration from such reactions could prove revolutionary as they provide us multiple dynamic systems and bifurcations which can be used in non-biological fields and perhaps even at small scales.

The results obtained here are not as important in their immediate effects as they are in giving to us a new path, a path to truly be able to describe biological activities through mathematical modeling and computer simulations. Such results may inspire scientists to walk down this thorny path and reach a state where all biology can be predicted and understood through simple equations, as such is the beauty of Non-linear Dynamics.

VIII. Open Questions

Where can we apply these features of biochemical oscillators is one of many unanswered problems. We are aware that in order to forecast motion in mechanical systems, their electrical counterparts can be studied. Could this analogy be applied to difficult to solve nonlinear dynamical biological systems? Additionally, it could have profound applications in the field of medical diagnosis and treatment. One can diagnose illnesses by computing the oscillatory equations of patients and contrasting them with ideal behaviour to come up with suitable treatment measures.

We can see that certain biochemicals give us very rich dynamics. Using this is it possible to devise machines and processes which use these chemicals or there derivatives to give us such rich dynamics? For example, using chemicals responsible for human biological clock, is it possible to devise a clock?

References

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