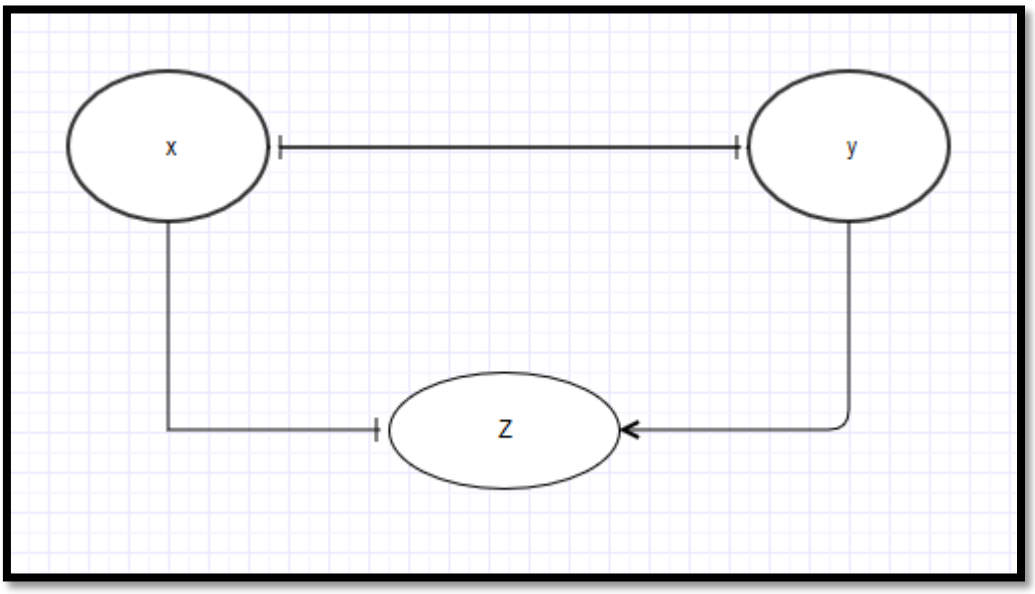
Homework 2 (chapters 3-6 of U. Alon "An introduction to Systems Biology").

Date: 2014-05-22

Part 1

Consider two genes which repress each other, $X \dashv Y$ and $Y \dashv X$, and which both regulate a third gene as $X \dashv Z$ and $Y \rightarrow Z$. Determine the steady states. Describe the responses to a change of the state variables (X and Y, one at a time), starting from a steady state.



Steady states:

In our case we have a double-negative feedback loop, which is made of two repression interactions. An output gene Z is regulated as shown in the figure. We have two steady states, for which either gene is ON in the double-negative loop. The following table sums it up.

	X	***Y***	***Z***
Steady-state 1	ON	OFF	OFF
Steady-state 2	OFF	ON	ON

In one stable state X is ON and Y is OFF, so that protein X represses Y expression and prevents it from being produced. The other stable state is the reverse: X is OFF and Y is ON. Thus, the double-negative loop expresses either X or Y. This is useful when genes regulated by X belong to different cell fates than the genes regulated by Y.

Responses:
The basic equation describing the evolution of a protein product X is:

$$\frac{dX}{dt} = f_X(Y) - a_X \cdot X \ : (1)$$

Therefore, to determine the steady states for, let's say X, we have to solve (1) for $\frac{dX}{dt} = 0$.
Now we have $X = X_{st}$ and we get:

$$X_{st} = \frac{f_X(Y)}{a_X} : (\delta)$$

Explaining the result: The higher the production rate $f_X(Y)$, the higher the protein concentration reached, X_{st}. The higher the rate of decay a_X, the lower is X_{st}.

Starting from the Steady-state 1, if we change the state variable X so that it's signal, let's say S_x, disappears and its production stops, we will get an exponential decay of X concentration. This is better understood if we solve equation (1) for X with $f_X(Y) = 0$.
Thus,

$$X(t) = X_{st} \cdot e^{-a_X \cdot t} : (\varepsilon)$$

A good measure for the speed at which X levels change is the **response time** i.e. the time to reach halfway between the initial and final levels in a dynamic process. We solve **(ε)** for $X_{st} = \frac{X_{st}}{2}$ and we get:

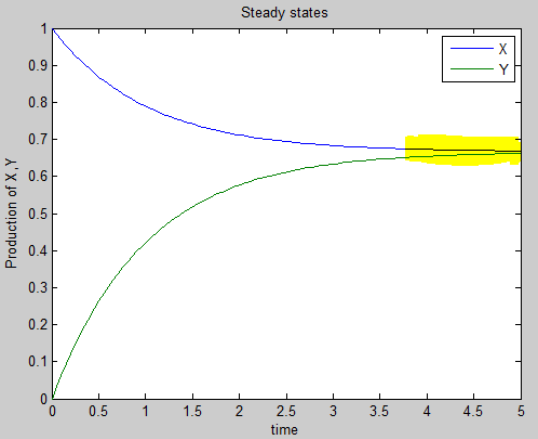
$$T_{response} = \frac{\log(2)}{a_X}$$

Now that Y is no longer repressed, given a signal S_y, it will begin to accumulate. The concentration of Y rises from zero and gradually converges on the steady-state $Y_{st} = \frac{f_Y(X)}{a_Y}$. According to the book, this convergence is shown in the following formula (which is derived from equation (1)):

$$Y(t) = Y_{st}(1 - e^{-a_Y \cdot t}) : (\zeta)$$

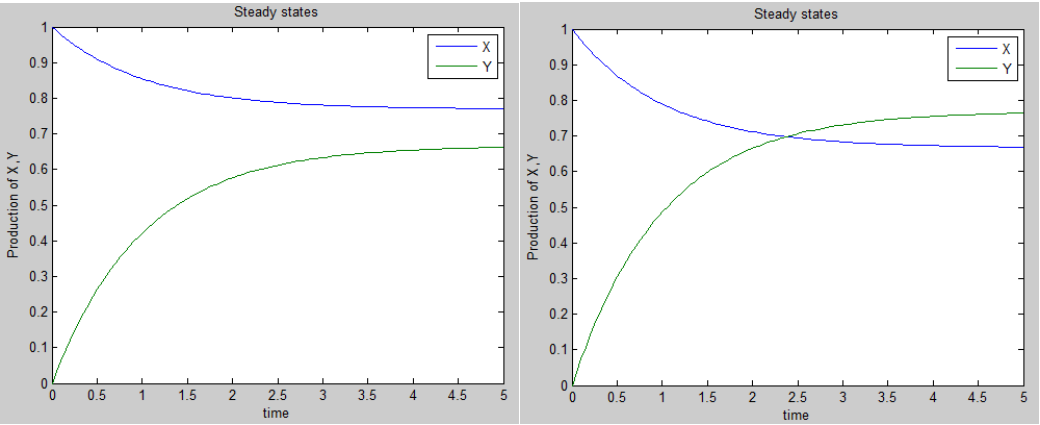
Moreover, the response time is $T_{response}$ once again.

In the opposite case, something similar happens. If a signal S_x suddenly appears, then X will begin to accumulate, in a similar manner to (ζ) and it will begin to repress Y, which in turn will see an exponential concentration decay similar to (ε).

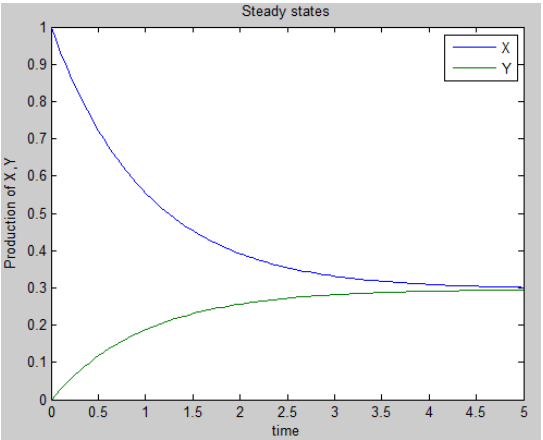


The highlighted areas on the curve denote the two **steady states**. For this model, the Hill coefficient n=1 was used.

Now by playing a bit with our variables (slowing production rate for X on the left figure and then for Y on the right one), we create a small finite perturbation and on the following graph we can see the system's response. It's obviously different which repressor's production rate we choose to diminish first.



Here I will also show the model with Hill coefficient 3.



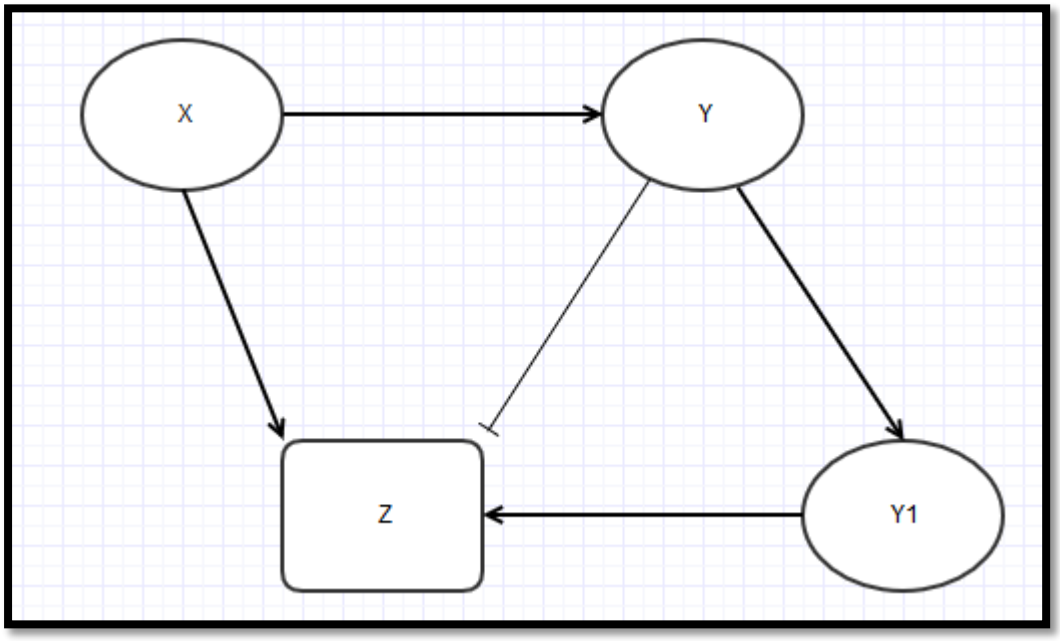
The larger the Hill coefficient, the steeper the curve.

*(**NOTE:** See Appendix in the end of the document for comments on the input functions)*

Part 2

Consider four genes X, Y, Y_1 and Z that interact as two interconnected feed-forward loops: $X \rightarrow Y, X \rightarrow Z$ and $Y \dashv Z$ such that (X,Y,Z) is an incoherent FFL of type I1 in Alon's classification (Fig 4.3 page 47); $Y \dashv Z, Y \rightarrow Y_1$ and $Y_1 \rightarrow Z$ such that (Y,Y_1,Z) is an incoherent FFL of type I3.
Consider as in the general set-up used by Alon that the three genes X, Y and Y_1 are driven by three input signals S_x, S_y and S_{y_1}. When the input signals are off (value 0) the corresponding protein is in its inactive form and cannot act as a transcription factor, even if present in high enough concentration. When on the other hand the signals are on (value 1) the corresponding protein is in its active form and can act as a transcription factor, if in high enough concentration.

The output node (Z) is now driven by three inputs: X, Y and Y_1. Choose one way of combining the inputs which makes Z a non-canalizing Boolean function of the inputs. We repeat from the lectures that a non-canalizing Boolean function is one which fully depends on all its arguments.
Describe precisely which Boolean function you chose and show that it is non-canalizing. Show how the expression of Z changes in response to steps in signals S_x, S_y and S_{y_1}. Comment.



I claim that the logical expression $\boldsymbol{X}$ *XOR* $\boldsymbol{Y}$ *XOR* $\boldsymbol{Y_1}$, which is a combination of the three inputs in our graph, makes Z a non-canalizing Boolean function of the inputs. It is non-canalizing since, none of the inputs can affect the outcome of the output individually, namely, our function is not canalizing towards any of the inputs. I show the truth table beside, as a simulation of the network. The value '1' should translate as "Protein's corresponding signal is present, thus the protein is in its active form" and the value '0' should denote the opposite.

X	Y_1	Y	X *XOR* Y *XOR* Y_1
0	0	0	**0**
1	0	0	**1**
0	1	0	**1**
0	0	1	**1**
1	1	0	**0**
1	0	1	**0**
0	1	1	**0**
1	1	1	**1**

Part 3

In this part it is sufficient to use logic input functions of the response of a downstream element to its net input. The net input should however be taken as a weighted sum of the upstream elements.

Consider a model of a three-level signal transduction pathway with two input signals (e.g. ligands) S_1 and S_2, two "MAKKK" kinases X_1 and X_2, two "MAPKK" kinases Y_1 and Y_2, two "MAPK"-level elements Z_1 and Z_2, and then an output element W, e.g. a transcription factor.
The net input to X_1 is u_1S_1 and the net input to X_2 is u_2S_2; the net input to Y_1 is $u_{11}X_1 + u_{12}X_2$, the net input to Y_2 is $u_{21}X_1 + u_{22}X_2$, the net input to Z_1 is $w_{11}Y_1 + w_{12}Y_2$, the net input to Z_2 is $w_{21}Y_1 + w_{22}Y_2$, and the net input to W is $r_1Z_1 + r_2Z_2$.
For the kinase X_1, the two weights w_{11} and w_{21} must all be non-negative, and similar for all the other kinases, while for a phosphatase all weights must be non-positive. The thresholds for activation/repression are K_{x_1} for X_1 etc. and the base-line dephosphorylation rates are a_{x_1} etc..
Suppose that the two "MAPK"-level elements Z_1 and Z_2 are kinases also.
Can you choose weights such that this three-layer perceptron computes the logical function XOR and EQ? If you cannot do so, can you give a reason why this is not possible?
Is the answer different if either or both of Z_1 and Z_2 act as a phosphatase?
Define what you consider as in- and outputs for these biochemical logical functions (e.g. what corresponds to TRUE and FALSE in your system) and try to use as few free parameters as possible.

Answer:
The XOR and EQ logical functions, both are complex functions which are not linearly separated by the perceptron in two dimensions. Therefore, they need to be approached by a multi-layer perceptron which is proved to create convex (more complex) decision surfaces.
The decision surface for XOR can be seen in Figure 1 (for EQ it is similar, perpendicular to XOR's) as the grey area between the two decision boundaries. In our case, we can see that there is no way to choose weights, for Z_1 and Z_2 acting as kinases, so that this three-layer perceptron computes the logical functions XOR or EQ. We need some inhibition, namely negative weights in our multi-layer perceptron, otherwise the weights will "push" our decision boundaries continuously and they will cover all our plane (the weights are the ones that position the decision boundaries in certain slopes/angles).

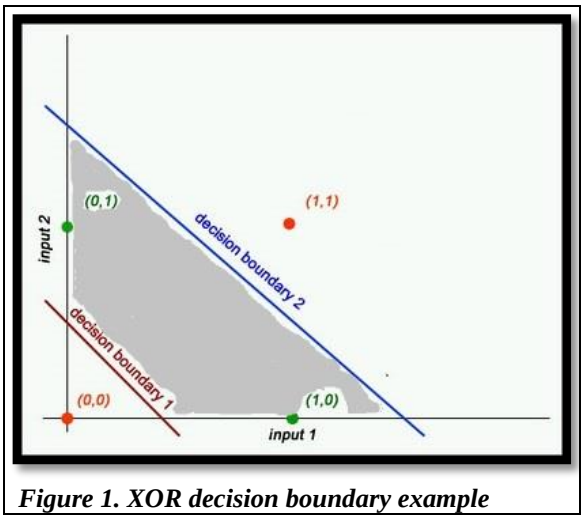


Figure 1. XOR decision boundary example

Just like the book on page 114, figure 6.13, we need a grey/active area for our output **W**, when either Z_1 or Z_2 are highly active, but not both, when talking about the XOR function. As far as the EQ function is concerned, we need a grey/active area for our output, when both Z_1 and Z_2 are active, but not when either of them is.
Summarizing, *for both Z1/Z2 as kinases, it's not possible to give weights. For one of them as phosphatase, we can find weights.*

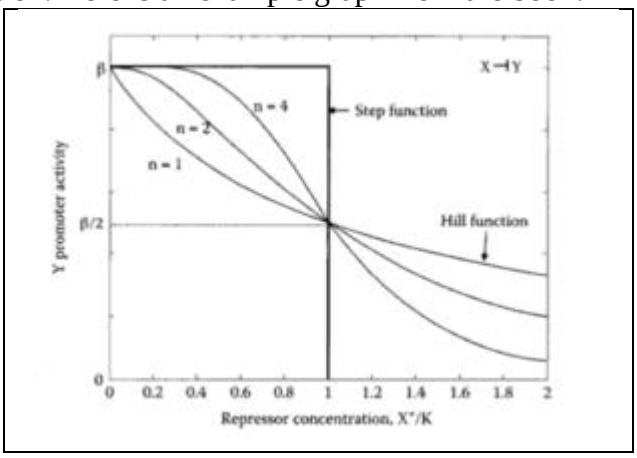
Note that, "TRUE" value denotes the fact that the weighted sum of the two input nodes is greater than a threshold value (which could be approximately 1) of the next layer's node to which they are inputs. "FALSE" means that the weighted sum did not overcome the threshold. In our case, by inputs we mean input kinase activities as well as the threshold, which is also discussed in terms of concentration of a phosphorylated kinase.

APPENDIX

Since we are talking about a repressor (X and Y form a negative feedback loop), we have the following models for our input functions:

Logic input function	Michaelis-Menten function	Cooperative function of Hill coefficient 3
$f_X(Y) = \begin{cases} \beta_X & \text{if } Y < K_{yx} \\ 0 & \text{if } Y > K_{yx} \end{cases}$	$f_X(Y) = \beta_X \frac{K_{yx}}{Y + K_{yx}}$	$f_X(Y) = \beta_X \frac{{K_{yx}}^3}{Y^3 + {K_{yx}}^3}$

The first model (Logic input), is often useful to show the essential behavior of the input functions and retain mathematical clarity, whereas the next two models are used to describe our input functions in more graded detail. The hill coefficient (n=3 for the third model) defines the steepness of the S-shaped function with high *n* resulting in more steep curves. If n=1, then we get the Michaelis-Menten function. Here is an example graph from the book:



The Hill function, in the limit when $n \to \infty$, becomes a step function, which is what the Logic Input function looks like graphically. Therefore, we could say that the logic input function is an approximation of graded functions like Hill functions.. The graded input functions show expression as soon as X* appears, whereas the logic input function shows expression only when X* crosses the threshold K. So, we conclude that we basically use the same function with an 'n-coefficient' (the dynamics are quite similar), which varies as to the level of detail in which we want to describe our system.