Exercise sheet

In this practical you will:

- Look at the effect of transcriptional leakiness on dose-response curves;
- Study feedback and autoregulation of transcriptional processes, and
- Observe time scale separation (as we did for the derivation of Michaelis-Menten) in another context.

Solutions will be made available on Brightspace.

Part 1: TRANSCRIPTION LEAKINESS

Leakiness is a term often used in synthetic biology to describe background levels of transcription that takes place unregulated by an activating or inhibiting transcription factor. This mechanism allows for a measure of control. For example, you want an inhibiting transcription factor to completely shut down transcription of a lethal compound. Another example is the background transcription of an enzyme that can deal with a certain substrate to allow for a quick response to when that substrate becomes available.

Let us take a look at the effect of leakiness on dose-response curves. We first consider a system in the absence of background transcription. Consider an inducer I that promotes transcription of A_m. In yesterday’s lecture we saw the following reactions:

$$\phi \xrightarrow{k_s \frac{I}{1+I}} A_m$$

$$A_m \xrightarrow{k_d} \phi$$

Q1: Write the above chemical reactions as one ODE.

Result: $\frac{dA_m}{dt} = k_s \frac{I}{1+I} - k_d A_m$

Q2: Solve this ODE to find the steady state of A_m, and explain from the expression why this steady state can ever be negative.

Steady state: $k_s \frac{I}{1+I} - k_d A_m = 0 \Rightarrow k_s \frac{I}{1+I} = k_d A_m \Rightarrow A_m = \frac{k_s}{k_d} \cdot \frac{I}{1+I}$

Because $I \geq 0$, and the reaction rates $k_s \geq 0$, $k_d \geq 0$, $A_m \geq 0$ always.

<u>Q3</u>: Set $k_s = k_d = 1$. Implement the function in Jupyter and plot $A_m = f(I)$ for $I = 0, I = 0.1$, and $I = 10$. Re-scale the x-axis using a logarithmic scale. (*Hint: The resulting curve should be S-shaped.*)

Resulting graph(s):

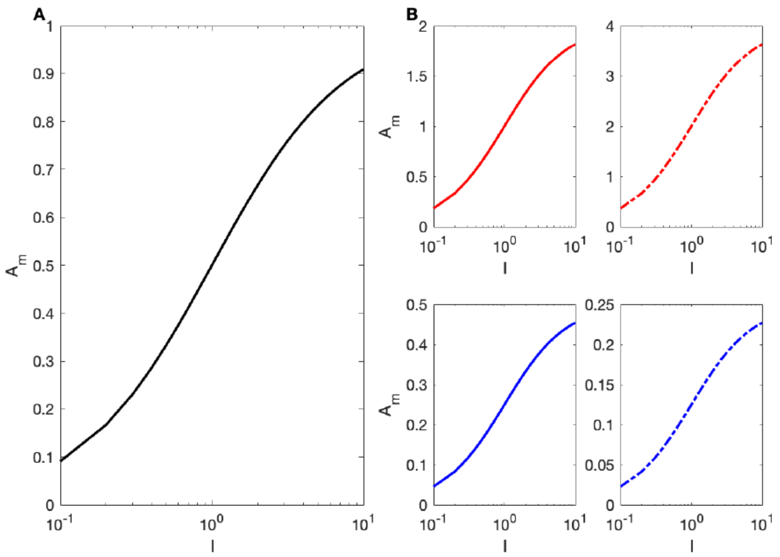


Figure 1: Panel A shows the solution with a logarithmic x-axis where $k_s = k_d = 1$. Panel B shows how the solution changes if k_s and k_d are changed. Top left: $(k_s, k_d) = (2, 1)$ or $(k_s, k_d) = (1, 0.5)$. Top right: $(k_s, k_d) = (2, 0.5)$. Bottom left: $(k_s, k_d) = (1, 2)$ or $(k_s, k_d) = (0.5, 1)$. Bottom right: $(k_s, k_d) = (0.5, 2)$.

Look at panel A.

<u>Q4</u>: Systematically vary k_s and k_d by dividing or multiplying each parameter by 2, either individually or both at the same time. What happens to the resulting dose-response curve?

Look at panel B. This should not be surprising, given $\frac{k_s}{k_d}$.

Yesterday we also added background translation – leakiness – to the chemical equations. This was depicted as:

$$\phi \xrightarrow{k_0 = 0.01} A_m$$

<u>Q5</u>: Modify the ODE from Q1 to include this reaction, and calculate the steady state.

Result: $\frac{dA_m}{dt} = k_0 + k_s \frac{I}{1+I} - k_d A_m$

Steady state: $k_0 + k_s \frac{I}{1+I} - k_d A_m = 0 \Rightarrow k_0 + k_s \frac{I}{1+I} = k_d A_m \Rightarrow A_m = \frac{k_0}{k_d} + \frac{k_s}{k_d} \cdot \frac{I}{1+I}$

<u>Q6</u>: Plot the dose-response curve for this modified model, and plot the curves for $k_0 = 0.1$ and $k_0 = 1$.

Result:

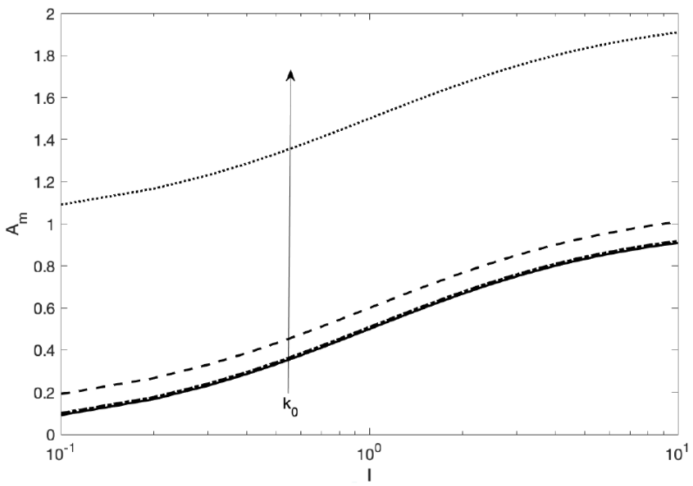


Figure 2: Dose-response curve of A_m against I for varying levels of background transcription k_0.

I.e., with increasing background transcription the curve is shifted upward. This is likely what you would expect: with $I = 0$ the production is fully determined by the leaky transcription.

Yesterday we also looked at how to assume inhibition rather than activation. Assume the added reaction is now:

$$\phi \xrightarrow{k_0 + k_s \frac{1}{1+I}} A_m$$

<u>Q7</u>: How does the dose-response curve change now? (*Hint: Modify the ODE, calculate the steady state, and implement the model in Jupiter like before*.)

Result:

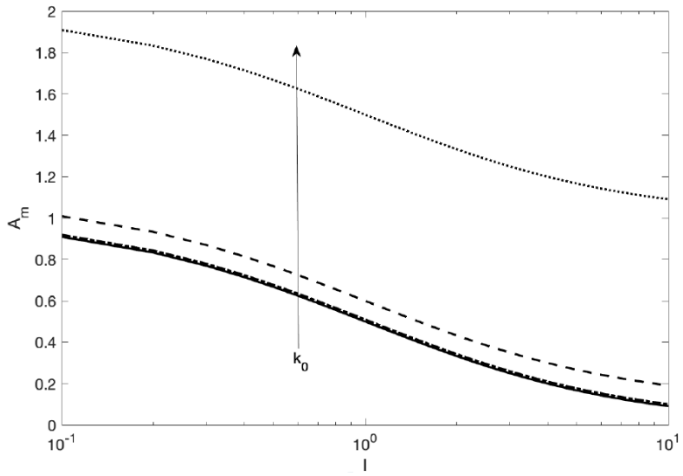


Figure 3: Dose-response curve of A_m against I for varying levels of background transcription k_0 with an inhibitory inducer.

It is essentially the mirrored image of the previous model result.

Part 2: AUTOREGULATION AND FEEDBACK

Now, we will replace the inducer of the previous exercise with a transcription factor. This way we can construct autoregulatory feedback loops on transcription. This is commonly referred to as a network motive; its biological mechanism is often found in nature, for instance, roughly half of the ca. 200 transcription factors in *E. coli* are autoregulated (Gao & Stock, 2018, DOI:10.1016/j.celrep.2018.08.023).

Given are the following reactions for positive autoregulation:

$$
\begin{aligned}
\phi &\xrightarrow{k_{sm}\frac{A_p}{1+A_p}} A_m \\
A_m &\xrightarrow{k_{dm}} \phi \\
A_m &\xrightarrow{k_{sp}} A_m + A_p \\
A_p &\xrightarrow{k_{dp}} \phi.
\end{aligned}
$$

Q8: Give the two ODEs for the above chemical equations for positive autoregulation.

First equation: $\frac{dA_m}{dt} = k_{sm}\frac{A_p}{1+A_p} - k_{dm}A_m - k_{sp}A_m + k_{sp}A_m = k_{sm}\frac{A_p}{1+A_p} - k_{dm}A_m$

Second equation: $\frac{dA_p}{dt} = k_{sp}A_m - k_{dp}A_p$

Q9: Assume $k_{sm} = k_{sp} = 2$, $k_{dm} = 0.1$, and $k_{dp} = 1$. Simulate the model in Jupyter with initial conditions $A_m(0) = A_p(0) = 0.1$ for a time span from $t_0 = 0$ to $t_0 = 50$. Does the system reach a steady state, and if yes, what is it? Plot the time series to show.

See the results of Q10 below.

Q10: Consider the same model with the same settings as in Q9, but with initial conditions $A_m(0) = A_p(0) = 60$. Does the system reach a steady state now, and if yes, what is it? Plot the time series to show.

Result:

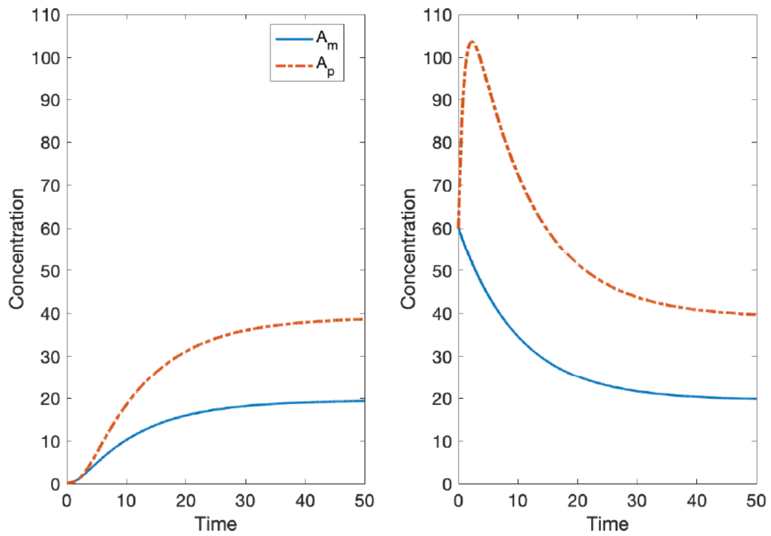


Figure 4: Time-series of A_m and A_p for (left) $A_m(0) = A_p(0) = 0.1$ and (right) $A_m(0) = A_p(0) = 60$.

So yes, with the other initial conditions the same steady state is reached.

Q11: Now create a plot in which the steady state value of A_p is a function of k_{dm}. Consider increasing values of k_{dm}, for instance, $0.15, 0.2, 0.25, 0.5$, and 1. Each time restart the simulations at $A_m(0) = A_p(0) = 60$. What happens to the steady state value of A_p as k_{dm} increases?

Result:

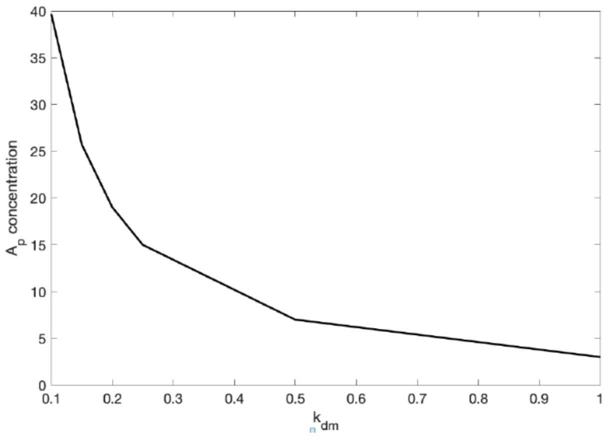


Figure 6: Plot of A_p concentration against mRNA degradation rate k_{dm}. The plot shows that as k_{dm} increases, the final concentration of A_p decreases.

Hence, A_p decreases as k_{dm} increases.

Q12: Show algebraically that the steady state of A_p is a function of k_{dm}. (*Hint: Follow the next steps: Substitute the numerical values of all parameters, except k_{dm}; Solve $\frac{dA_m}{dt} = 0$ to get $A_m = f(A_p)$; Solve $\frac{dA_p}{dt} = 0$ to get $A_p = g(A_m)$; Substitute $f(A_p)$ into $A_p = g(A_m)$ to get $A_p = h(A_p)$, then rearrange and solve.*)

Substitute parameter values: $\frac{dA_m}{dt} = 2\frac{A_p}{1+A_p} - k_{dm}A_m$; $\frac{dA_p}{dt} = 2A_m - A_p$

Second hint: $2\frac{A_p}{1+A_p} - k_{dm}A_m = 0 \Rightarrow 2\frac{A_p}{1+A_p} = k_{dm}A_m \Rightarrow A_m = \frac{2}{k_{dm}} \cdot \frac{A_p}{1+A_p}$

Third hint: $2A_m - A_p = 0 \Rightarrow A_p = 2A_m$

Fourth hint: $A_p = 2A_m = 2\left(\frac{2}{k_{dm}} \cdot \frac{A_p}{1+A_p}\right) = \frac{4}{k_{dm}} \cdot \frac{A_p}{1+A_p}$

Rearranging: $A_p = \frac{4}{k_{dm}} \cdot \frac{A_p}{1+A_p} \Rightarrow A_p(1 + A_p) = \frac{4A_p}{k_{dm}} \Rightarrow A_p(1 + A_p) - \frac{4A_p}{k_{dm}} = A_p\left((1 + A_p) - \frac{4}{k_{dm}}\right) = 0$

This gives $A_p = 0$ (biologically possible) or $(1 + A_p) - \frac{4}{k_{dm}} = 0 \Rightarrow 1 + A_p = \frac{4}{k_{dm}} \Rightarrow A_p = \frac{4}{k_{dm}} - 1$

As k_{dm} is in the denominator, increasing it will decrease A_p, i.e., the steady state.

While the previous was an example with feedback involving a single component, many feedback loops in biological systems involve two or more components. Consider the following system with the reactions:

$$\begin{aligned}
\phi &\xrightarrow{k_{sm1}\frac{1}{1+B_p}} A_m \\
A_m &\xrightarrow{k_{dm1}} \phi \\
A_m &\xrightarrow{k_{sp1}} A_m + A_p \\
A_p &\xrightarrow{k_{dp1}} \phi. \\
\phi &\xrightarrow{k_{sm2}\frac{A_p}{1+A_p}} B_m \\
B_m &\xrightarrow{k_{dm2}} \phi \\
B_m &\xrightarrow{k_{sp2}} B_m + B_p \\
B_p &\xrightarrow{k_{dp2}} \phi.
\end{aligned}$$

Q13: Give the ODEs of this system and simulate the system from $t_0 = 0$ to $t_0 = 100$, setting $k_{sm1} = k_{sm2}1$, $k_{dm1} = k_{dm2} = 0.1$, $k_{sp1} = k_{sp2}0.25$, $k_{dp1} = k_{dp2} = 0.5$, and as initial conditions all variables set to 1. Describe the type of behaviour you see (*Remark: We will discuss this behaviour more in-depth in the coming weeks*.)

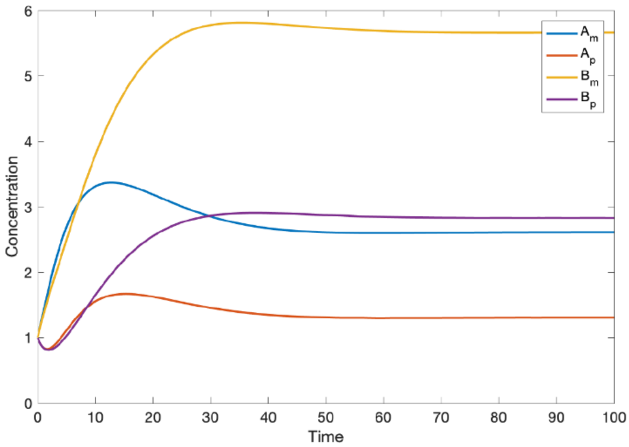
First equation: $\frac{dA_m}{dt} = k_{sm1}\frac{1}{1+B_p} - k_{dm1}A_m - k_{sp1}A_m + k_{sp1}A_m = k_{sm1}\frac{1}{1+B_p} - k_{dm1}A_m$

Second equation: $\frac{dA_p}{dt} = k_{sp1}A_m - k_{dp1}A_p$

Third equation: $\frac{dB_m}{dt} = k_{sm2}\frac{A_p}{1+A_p} - k_{dm2}B_m - k_{sp2}B_m + k_{sp2}B_m = k_{sm2}\frac{A_p}{1+A_p} - k_{dm2}B_m$

Fourth equation: $\frac{dB_p}{dt} = k_{sp2}B_m - k_{dp2}B_p$

The students should see dampened oscillations:



This will come back in the next weeks.

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