Project 5: The Repressilator

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Introduction

The repressilator is a famous early experiment in synthetic biology, in which scientists Elowitz and Leibler successfully built a biological system not found anywhere in nature. They combined genes from three different organisms into one system where each gene represses the expression of the next, much like a game of rock-paper-scissors. This negative feedback loop acts like a predictable clock: the levels of protein oscillate in a cycle. When a fluorescent protein is added to the system, you can actually see pulsing lights in the bacterial cell under the microscope. Here are some cool (sped-up) videos of fluorescing bacteria: https://biocircuits.github.io/chapters/09_repressilator.html#Will-it-work?

Elowitz and Leibler formulated a dynamical system to model the repressilator, which we explore here. Here is the system (from Ellner and Guckenheimer Eqn. 4.1):

$$\dot{m}_i = -m_i + \frac{\alpha}{1 + p_j^n} + \alpha_0$$

$$\dot{p}_i = -\beta(p_i - m_i).$$

m_i = concentration of mRNA i
p_i = concentration of protein i

For the second equation: The amount of protein over time depends on how quickly mRNA is converted into protein (production rate, $B*m_i$) and how quickly the protein decays (decay rate, $-B*p_i$).

The first equation is more interesting. The decay rate ($-m_i$) is simple, but the production rate is complicated. The rock-paper-scissors nature of the system means that the amount of mRNA which is being produced depends on how much of a *different* protein there is. That is because the three proteins function as repressors: they repress the production of mRNA for their neighbor. Protein 1 represses production of mRNA 2, protein 2 represses production of mRNA 3, and protein 3 represses production of mRNA 1. So to calculate the rate of production of mRNA i, we need to look at the amount of protein j. There is a base transcription rate (a0) which doesn't depend on the protein concentration, plus another term ($a/(1+p_j\text{^}n)$) which does.

The system will only oscillate if the conditions are right, depending on parameters. a0, a, b, and n are the parameters, which we explore in the results section.

Results

Legend for all figures:

mRNA:	Protein:
Red = mlacI	LightBlue = placI
DarkBlue = mtetR	Magenta = ptetR
LightGreen = mcI	DarkGreen = pcI

Baseline mRNA transcription rate with repression (a0)

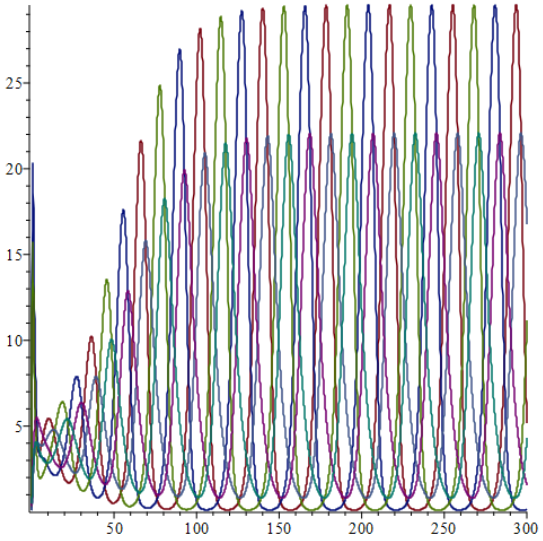

Figure 1: a0 = 0 (no baseline transcription)

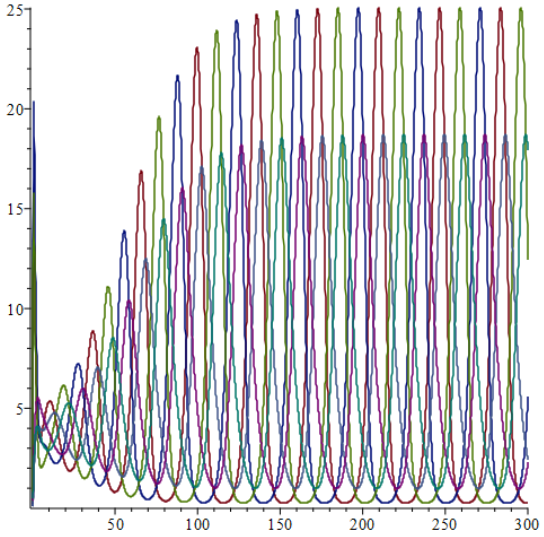

Figure 2: a0 = 0.1 (small baseline transcription)

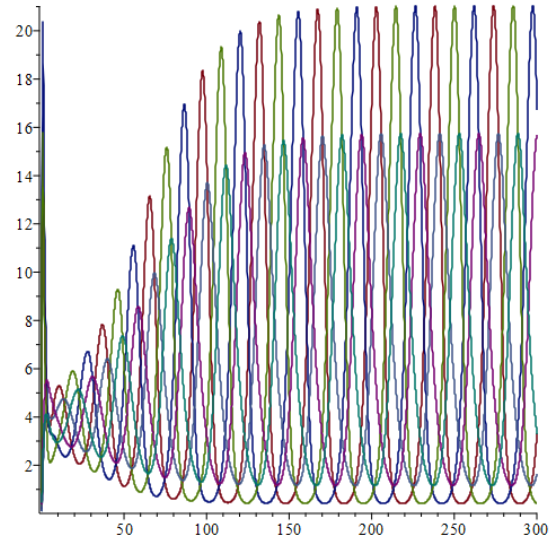

Figure 3: a0 = 0.2 (moderate baseline)

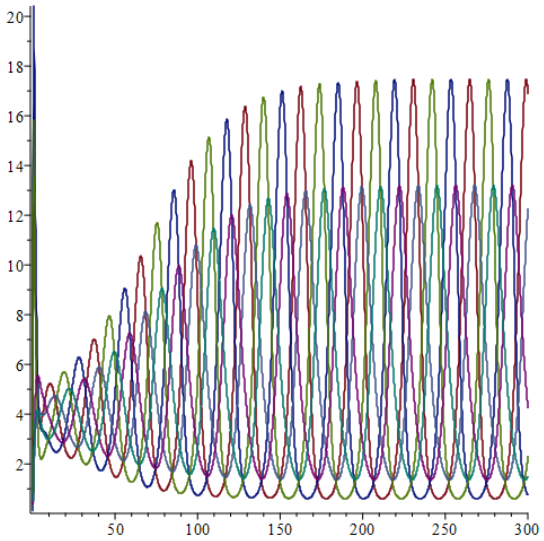

Figure 4: a0 = 0.3 (high baseline)

Additional mRNA transcription rate with no repression (a)

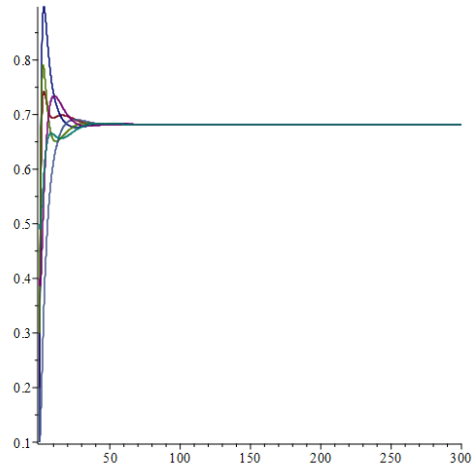

Figure 5: a = 1 (very low transcription)

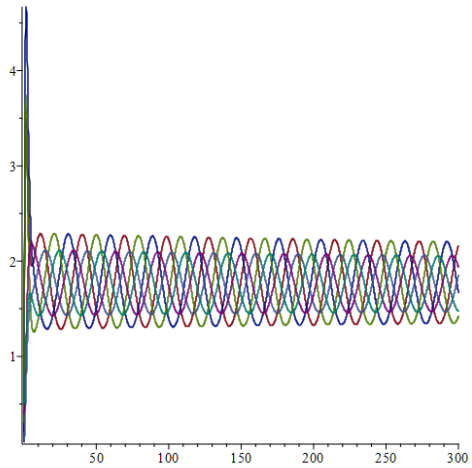

Figure 6: a = 7 (just below critical threshold)

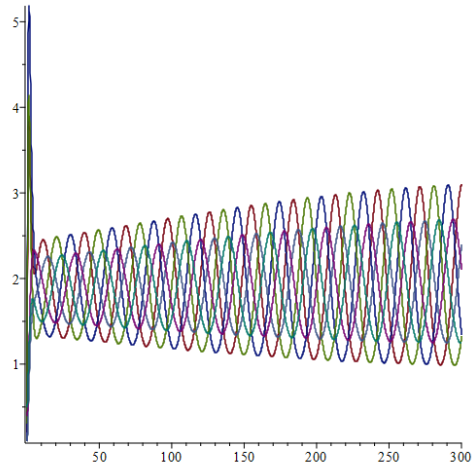

Figure 7: a = 8 (just above critical threshold)

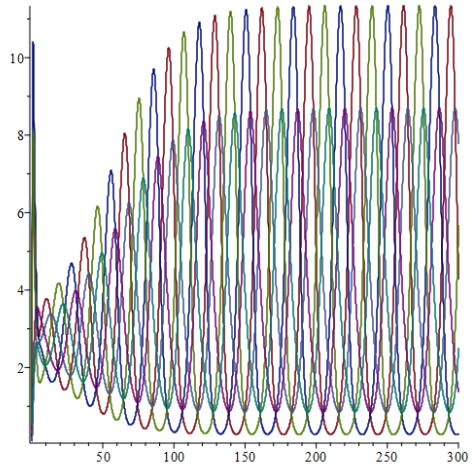

Figure 8: a = 20 (moderate transcription rate)

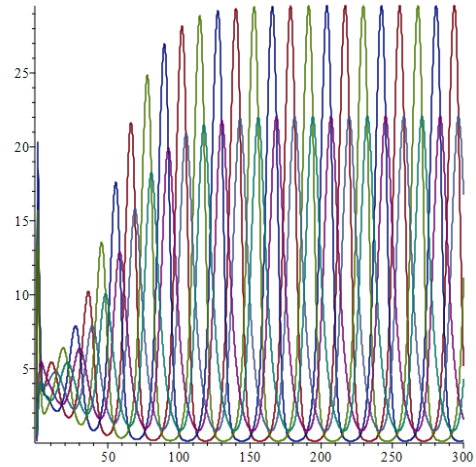

Figure 9: a = 50 (high transcription)

Rate of protein decay divided by rate of mRNA decay (b)

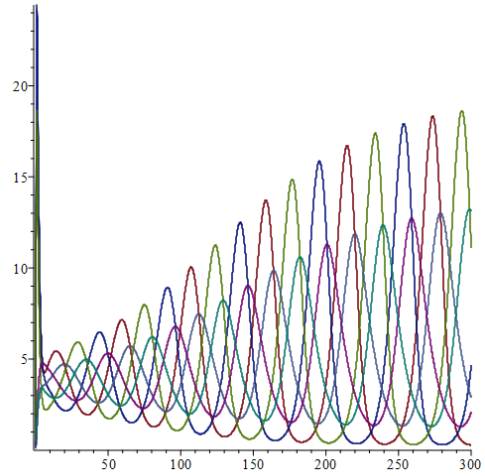

Figure 10: b = 0.1 (slow protein decay - longer period)

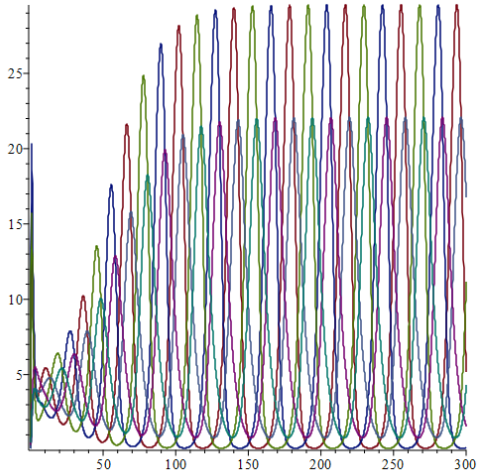

Figure 11: b = 0.2 (baseline rate)

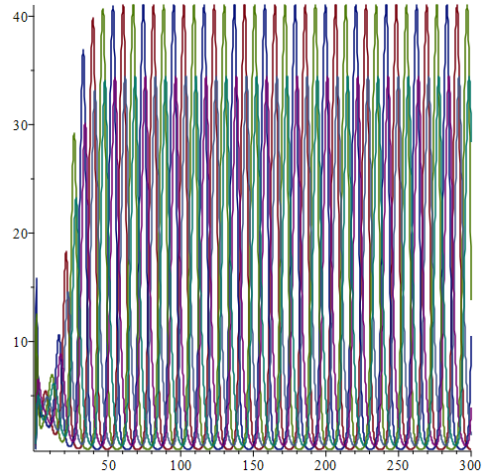

Figure 12: b = 0.5 (moderate protein decay)

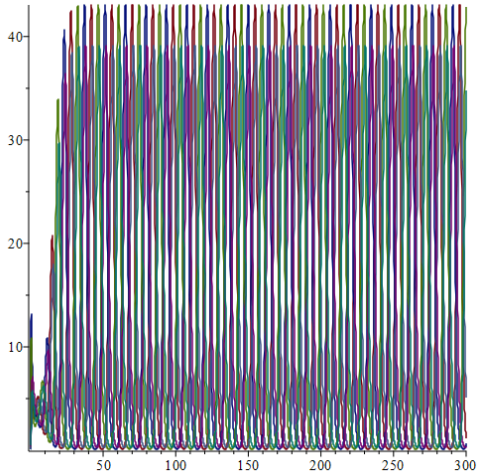

Figure 13: b = 1.0 (fast protein decay - shorter period)

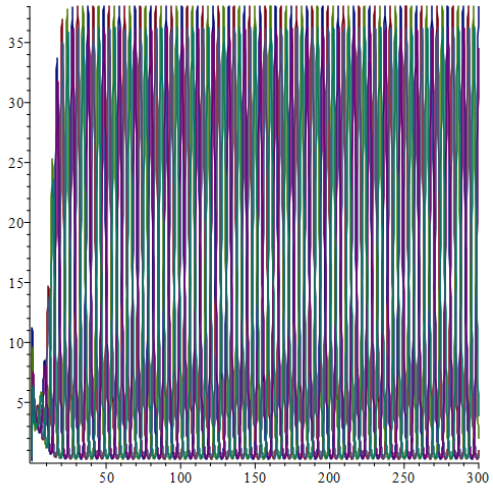

Figure 14: b = 2.0 (very fast decay)

Hill coefficient (n)

This coefficient represents how quickly the repressor “turns off” the production of its target mRNA.

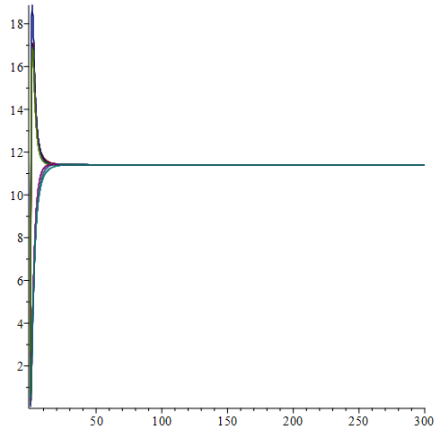

Figure 15: n = 0.5 (weak cooperativity)

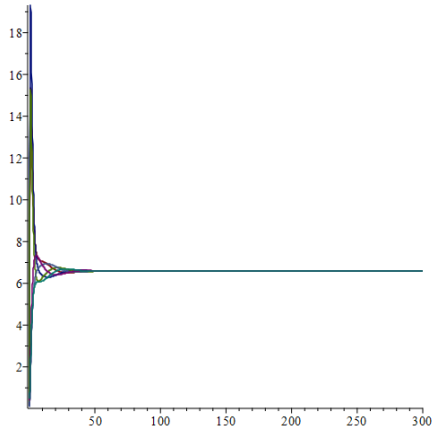

Figure 16: n = 1 (linear response - no cooperativity)

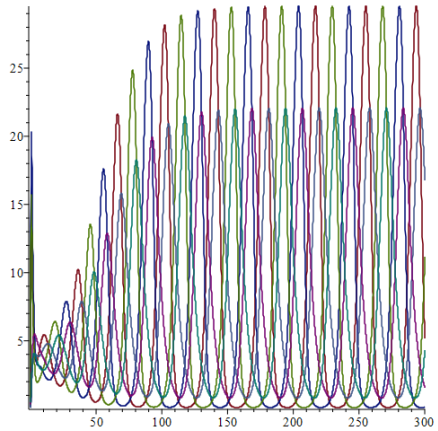

Figure 17: n = 2 (moderate cooperativity - baseline)

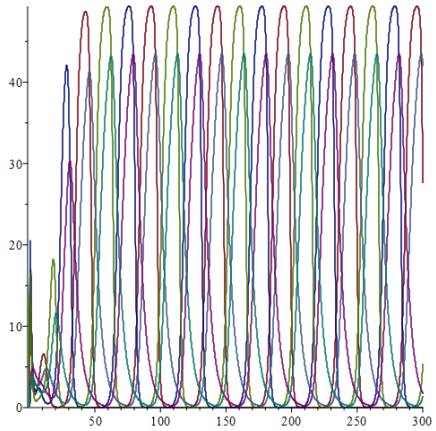

Figure 18: Case 4: n = 3 (strong cooperativity)

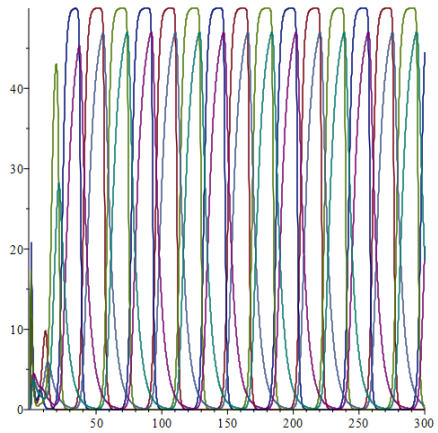

Figure 19: n = 4 (very strong cooperativity - sharp switch)

Discussion

If we were designing this system, how should we adjust the parameters to ensure that the system oscillates (interesting!) instead of reaching a stable equilibrium (boring)?

Baseline mRNA transcription rate with repression (a0): Keep a0 as low as possible, ideally at or near zero. When a0 = 0 (Figure 1), we observe robust periodic oscillations where the three proteins take turns repressing each other in a clear rock-paper-scissors pattern. As a0 increases to 0.1 (Figure 2), oscillations persist but with slightly reduced amplitude. However, when a0 reaches 0.2-0.3 (Figures 3-4), the oscillations become heavily damped or disappear entirely, with the system settling into a stable equilibrium. This makes biological sense: if there's always some "leaky" baseline transcription happening regardless of repression, it undermines the negative feedback loop that drives the oscillator. The repressor proteins can't fully shut off their targets, weakening the cyclic dynamics.

Additional mRNA transcription rate with no repression (a): Use a high value, well above the critical threshold of approximately 7-8. At a = 1 (Figure 5), the system immediately converges to a stable equilibrium with no oscillations, the transcription rate is simply too weak to maintain the feedback loop. At a = 7 (Figure 6), we're just below the bifurcation point and still see convergence to equilibrium. The magic happens at a = 8 (Figure 7), where oscillations suddenly emerge as we cross the critical threshold. As a increases further to 20 (Figure 8) and 50 (Figure 9), the oscillations become increasingly robust and regular. This parameter essentially controls the "strength" of the repression; you need strong transcription (when unrepressed) to create dramatic enough differences between the repressed and unrepressed states for oscillations to occur.

Rate of protein decay divided by rate of mRNA decay (b): The system tolerates a range of b values while maintaining oscillations, though b affects the period and amplitude. At b = 0.1 (Figure 10), oscillations occur with longer periods, protein levels change more slowly because decay is slow. At the baseline b = 0.2 (Figure 11), we see regular oscillations. As b increases to 0.5, 1.0, and 2.0 (Figures 12-14), oscillations persist but with progressively shorter periods and different amplitudes. The faster the protein decays relative to mRNA, the quicker the oscillation cycles. For a functioning repressilator, moderate values (b = 0.2-0.5) seem optimal, but the system is relatively robust to changes in this parameter compared to a0 and a.

Hill coefficient (n): Higher values of n produce more robust oscillations by making repression more switch-like. At n = 0.5 (Figure 15), repression is gradual and weak, leading to damped oscillations or convergence. With n = 1 (Figure 16), we have a linear response without cooperativity, and oscillations are weak or absent. At the baseline n = 2 (Figure 17), we observe clear periodic behavior. As n increases to 3 and 4 (Figures 18-19), the oscillations become even sharper and more pronounced. The Hill coefficient determines how "steep" the repression curve is; higher cooperativity means that as the repressor protein concentration increases, it more dramatically shuts down its target. This creates the strong nonlinearity necessary for sustained oscillations. For synthetic biology applications, $n \geq 2$ is desirable.

References

Original repressilator paper:
Elowitz, M., and S. Leibler. 2000. A synthetic oscillatory network of transcriptional regulators. Nature 403: 335–338

Textbook chapter:
Stephen P. Ellner and John Guckenheimer. Chapter 4: Cellular Dynamics: Pathways of Gene Expression from *Dynamic Models in Biology.* https://www.jstor.org/stable/j.ctvcm4h1q.9

Explanation of repressilator:
https://www.asimov.press/p/gene-circuit

Cool videos of bacteria fluorescing in cycles:
https://biocircuits.github.io/chapters/09_repressilator.html

Appendix

```
#Figures 1-4: PARAMETER a0 (Baseline mRNA transcription rate)
print(`Baseline mRNA transcription rate with repression`);
print(`Fixed parameters: a=50, b=0.2, n=2`);
print(`Case 1: a0 = 0 (no baseline transcription)`);
PlotGeneNet(0, 50, 0.2, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 2: a0 = 0.1 (small baseline transcription)`);
PlotGeneNet(0.1, 50, 0.2, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 3: a0 = 0.2 (moderate baseline)`);
PlotGeneNet(0.2, 50, 0.2, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 4: a0 = 0.3 (high baseline)`);
PlotGeneNet(0.3, 50, 0.2, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(``);

#Figures 5-9: PARAMETER a (Additional mRNA transcription rate)
print(`Additional mRNA transcription rate with no repression`);
print(`Fixed parameters: a0=0, b=0.2, n=2`);
print(`Case 1: a = 1 (very low transcription, similar to Fig 4.3)`);
PlotGeneNet(0, 1, 0.2, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 2: a = 7 (just below critical threshold)`);
PlotGeneNet(0, 7, 0.2, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 3: a = 8 (just above critical threshold)`);
PlotGeneNet(0, 8, 0.2, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 4: a = 20 (moderate transcription rate)`);
PlotGeneNet(0, 20, 0.2, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 5: a = 50 (high transcription, similar to Fig 4.2)`);
PlotGeneNet(0, 50, 0.2, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(``);
```

```
#Figures 10-14: PARAMETER b (Protein/mRNA decay rate ratio)
print(`Rate of protein decay divided by rate of mRNA decay`);
print(`Fixed parameters: a0=0, a=50, n=2`);
print(`Case 1: b = 0.1 (slow protein decay - longer period)`);
PlotGeneNet(0, 50, 0.1, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 2: b = 0.2 (baseline rate)`);
PlotGeneNet(0, 50, 0.2, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 3: b = 0.5 (moderate protein decay)`);
PlotGeneNet(0, 50, 0.5, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 4: b = 1.0 (fast protein decay - shorter period)`);
PlotGeneNet(0, 50, 1.0, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 5: b = 2.0 (very fast decay)`);
PlotGeneNet(0, 50, 2.0, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(``);

#Figures 15-19: PARAMETER n (Hill coefficient)
print(`Hill coefficient - cooperativity of repression`);
print(`Fixed parameters: a0=0, a=50, b=0.2`);
print(`Case 1: n = 0.5 (weak cooperativity)`);
PlotGeneNet(0, 50, 0.2, 0.5, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 2: n = 1 (linear response - no cooperativity)`);
PlotGeneNet(0, 50, 0.2, 1, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 3: n = 2 (moderate cooperativity - baseline)`);
PlotGeneNet(0, 50, 0.2, 2, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 4: n = 3 (strong cooperativity)`);
PlotGeneNet(0, 50, 0.2, 3, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
print(`Case 5: n = 4 (very strong cooperativity - sharp switch)`);
PlotGeneNet(0, 50, 0.2, 4, [0.2, 0.1, 0.3, 0.1, 0.4, 0.5], 0.1, 300);
```