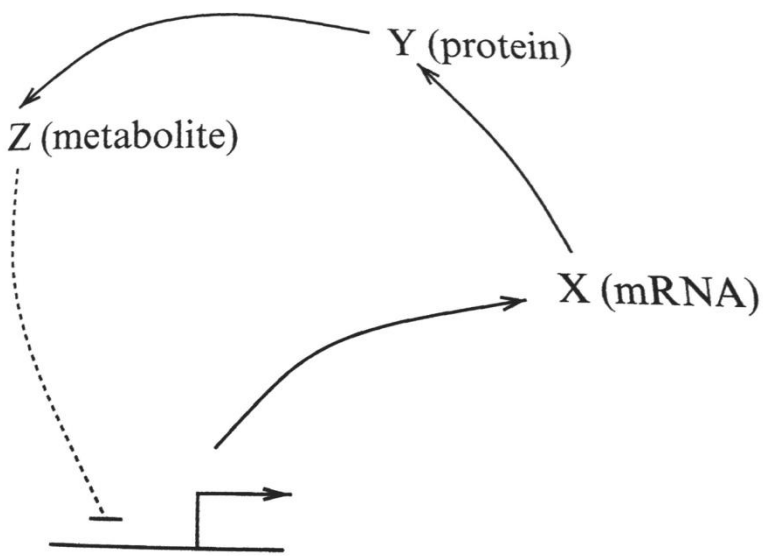
BME 5742

Genetic Oscillators

Biological Clocks

- Simple gene regulatory networks can generate persistent oscillations.
- Such networks allow cells to maintain internal clocks that are responsible for periodic changes in conditions.
- Example: Circadian Rhythms (such as sleep-wake cycles)

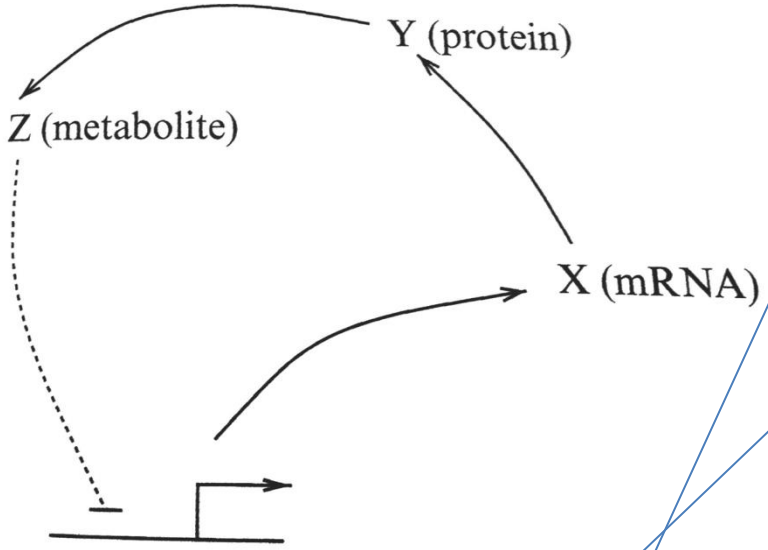
Goodwin's Oscillator



The specifics of catalysis and inhibition are neglected

- A generic model proposed in 1965
- Model involves a single gene.
- X denotes the mRNA.
- X is translated into enzyme Y.
- Y catalyzes production of a metabolite Z.
- Z inhibits the expression of X by activating some un-modeled repressor.

Goodwin's Oscillator Proposed Mathematical Model



$$\frac{dx(t)}{dt} = \frac{a}{k^n + (z(t))^n} - bx(t)$$

$$\frac{dy(t)}{dt} = \alpha \cdot x(t) - \beta \cdot y(t)$$

$$\frac{dz(t)}{dt} = \gamma \cdot y(t) - \delta \cdot z(t)$$

Linear translation and catalyzing models; Standard Hill function inhibition model

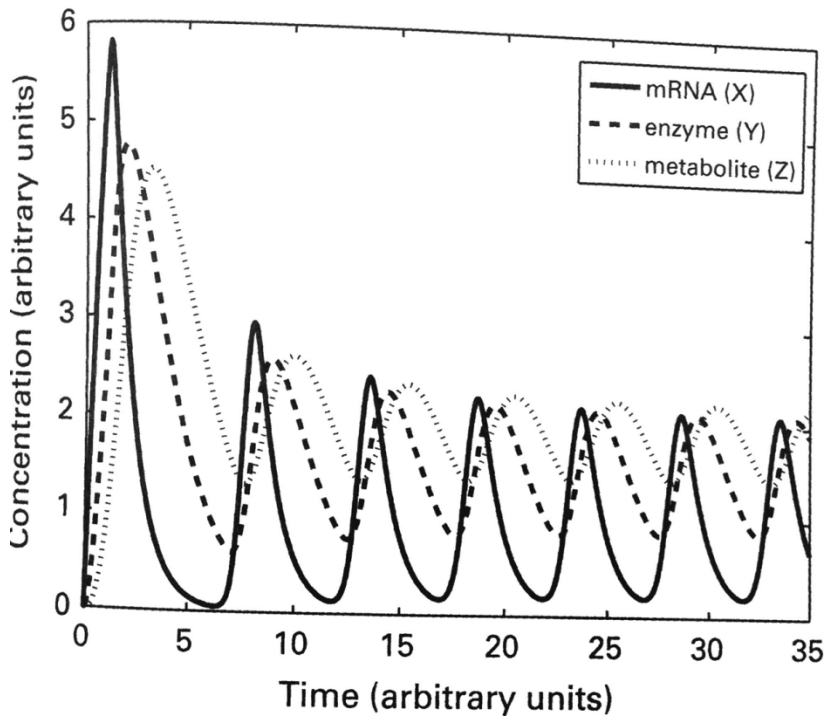
Comments about Goodwin's Oscillator

- No attempt was ever made (most likely) to fit the model to any physiological real data
- It was proposed just to demonstrate that persistent oscillations could be generated (under certain condition) by an auto-inhibitory gene circuit.
- The model has been analyzed by simulations.
- Goodwin's intuition was that at least three states are needed to create sufficient delay in the loop

Comments about Goodwin's Oscillator

- It is known in many control systems that oscillations can arise from negative feedback if the effect of the feedback is delayed and if there is sufficient nonlinearity in the loop.
- Studies done by J.S. Griffith (1968) confirmed that a two-state model (one without the metabolite Z) cannot possibly oscillate.

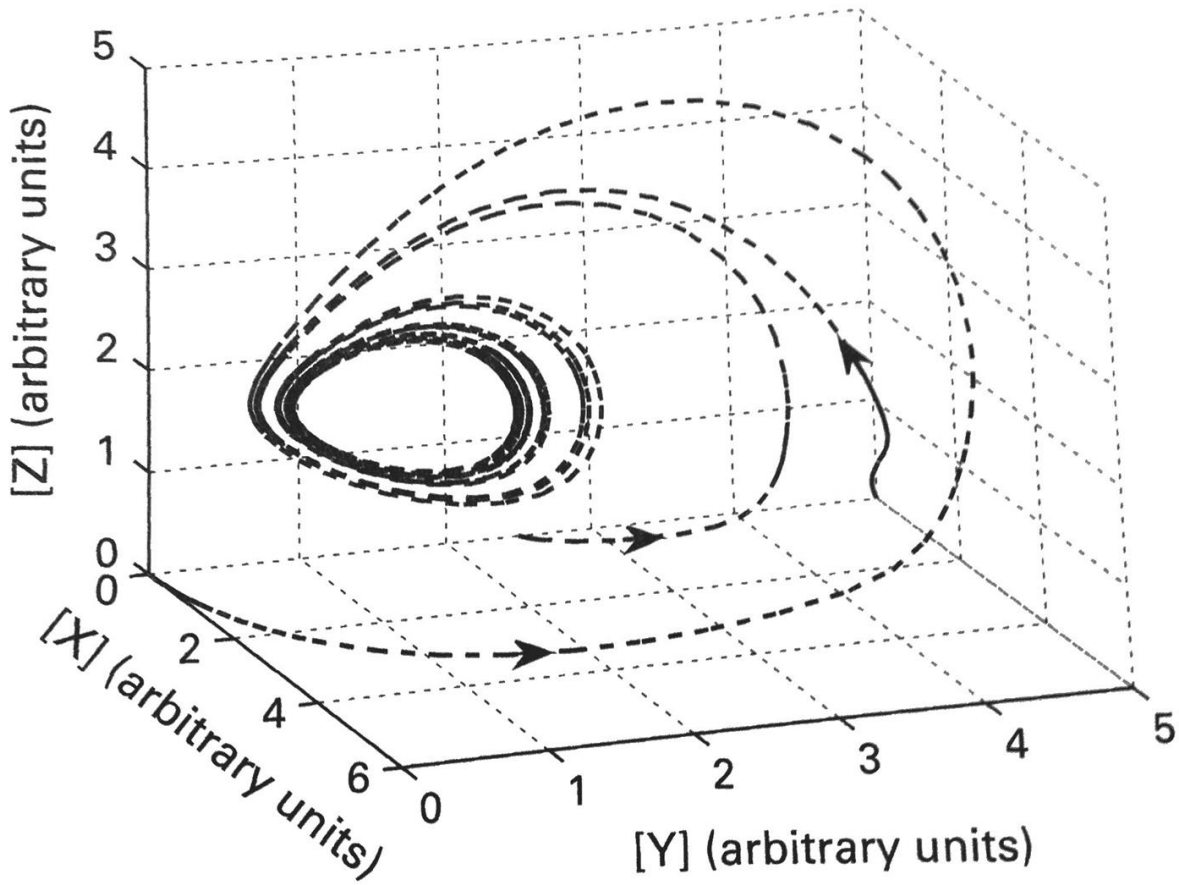
Goodwin's Oscillator Simulation Results



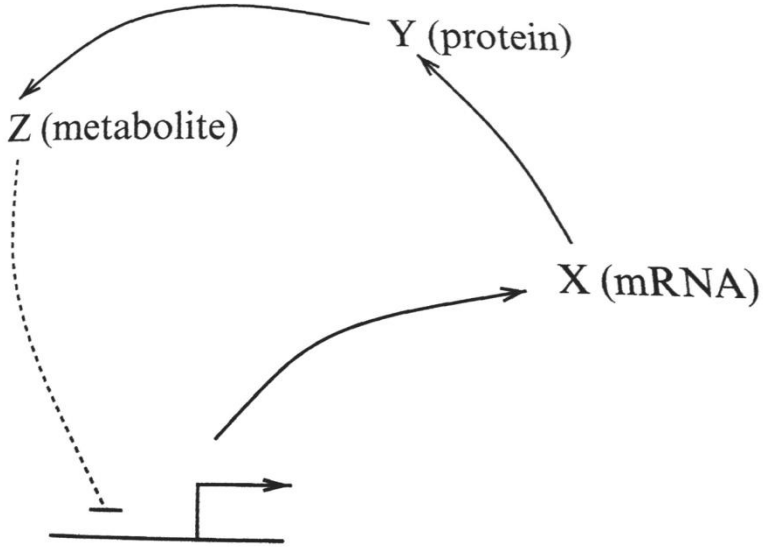
Limit cycle with period of approximately 4 time units

- Parameter values are: a = 360 (conc.·time^{-1}), k =1.368 (conc.), b = 1 (time^{-1}), α = 1 (time^{-1}), β = 0.6 (time^{-1}), γ = 1 (time^{-1}), δ = 0.8 (time^{-1}), n = 12.
- As x rises, y rises and then z rises, followed by crash of x, causing y and z to drop, and x rises again.

A different view of the oscillations shown in the previous example



Goodwin's Oscillator: How large should the Hill coefficient n be?



$$\frac{dx(t)}{dt} = \frac{a}{k^n + (z(t))^n} - bx(t)$$

$$\frac{dy(t)}{dt} = \alpha \cdot x(t) - \beta \cdot y(t)$$

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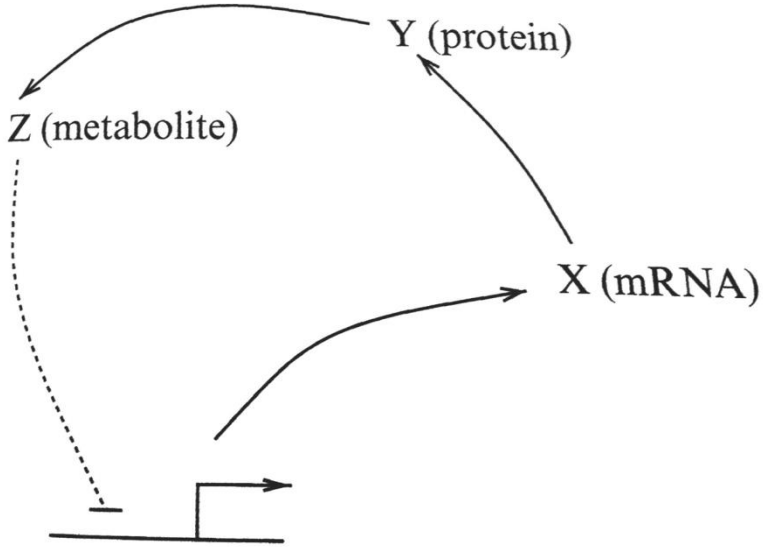
For many reasonable parameters, n must be at least 8!

Goodwin's Oscillator: Can n that assures oscillations be lower than 8?



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If a fourth state is added by adding a second metabolite → n < 8

That is, Z creates W linearly, and W is the repressor

Goodwin's Oscillator: Can n that assures oscillations be lower than 8?

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$$\frac{dy(t)}{dt} = \alpha \cdot x(t) - \beta \cdot y(t)$$

$$\frac{dz(t)}{dt} = \gamma \cdot y(t) - \frac{\delta \cdot z(t)}{K_M + z(t)}$$

$$\cancel{\frac{dx(t)}{dt} = \frac{a}{k^n + (z(t))^n} - bx(t)}$$

$$\cancel{\frac{dy(t)}{dt} = \alpha \cdot x(t) - \beta \cdot y(t)}$$

$$\cancel{\frac{dz(t)}{dt} = \gamma \cdot y(t) - \delta \cdot z(t)}$$

Degradation of Z modeled as Michaelis-Menten → Some parameter combinations create sustained oscillation even if n = 1! (that is, no cooperativity in the transcription)

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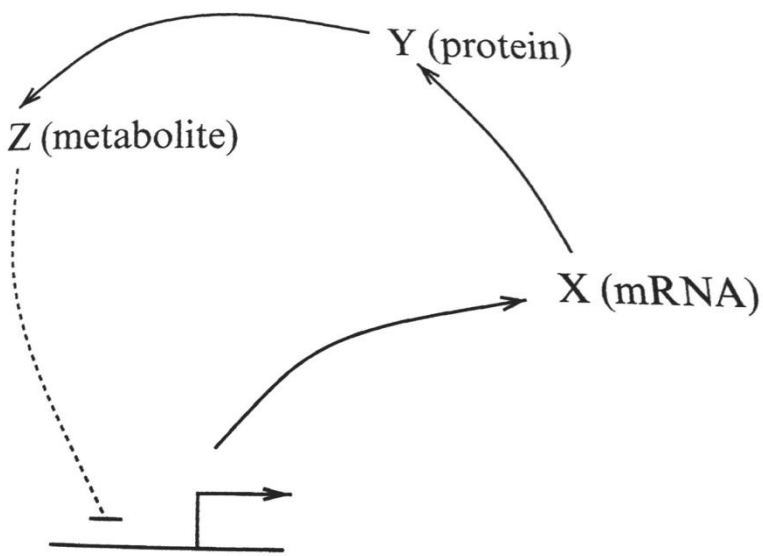
- Parameter values are: a = 150 (conc.·time^{-1}), k =1 (conc.), b = 0.2 (time^{-1}), α = 0.2 (time^{-1}), β = 0.2 (time^{-1}), γ = 0.2 (time^{-1}), δ = 15 (time^{-1}), n = 1, K_M = 1

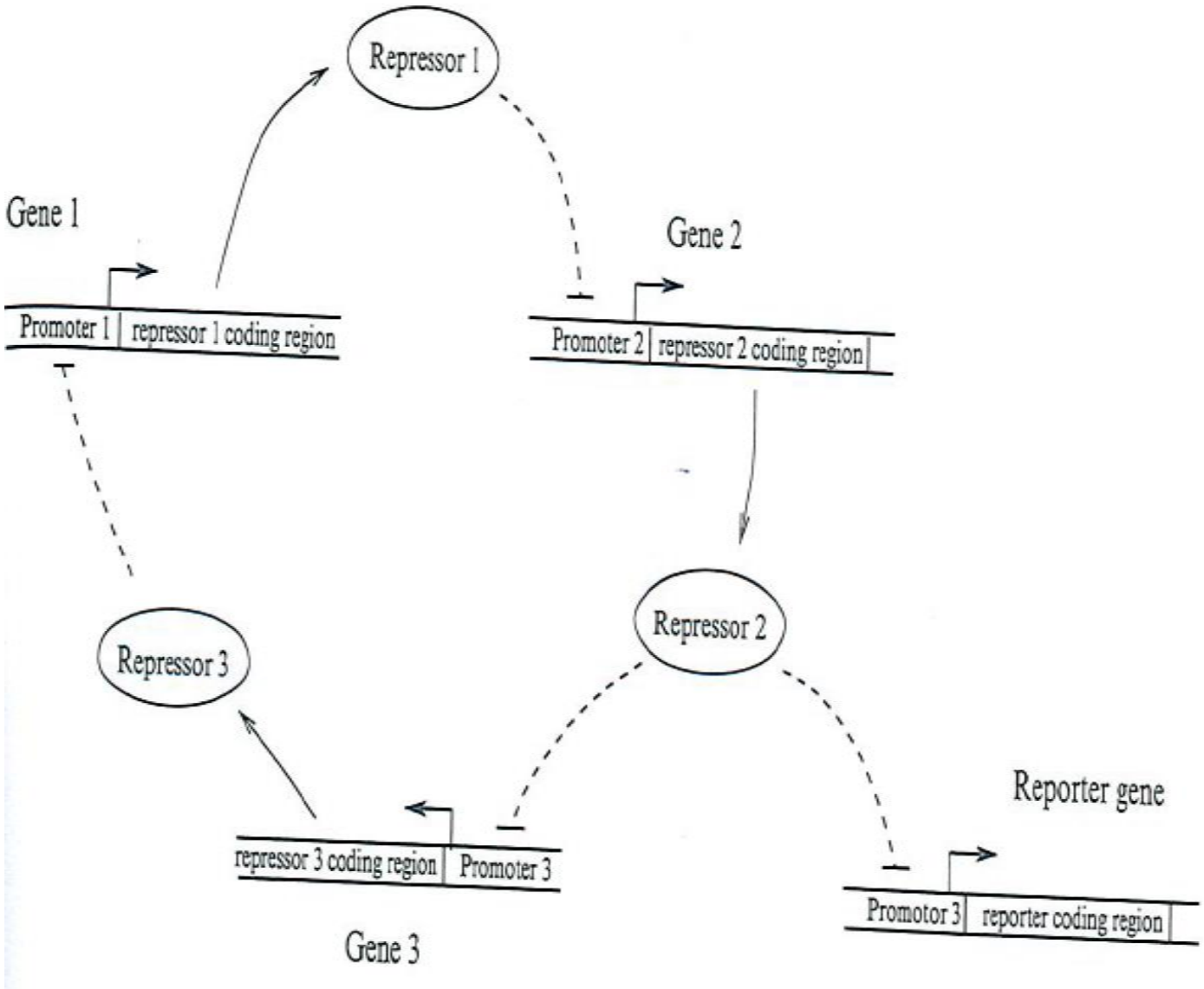
Degradation of Z modeled as Michaelis-Menten → Some parameter combinations create sustained oscillation even if n = 1! (that is, no cooperativity in the transcription)

Goodwin's Oscillator – Second Possible Interpretation



The specifics of catalysis and inhibition are neglected

- Model involves a single gene.
- X is nuclear mRNA.
- Y is cytoplasmic mRNA
- Z is a repressor protein product
- Z inhibits the expression of X over multiple operator sites.

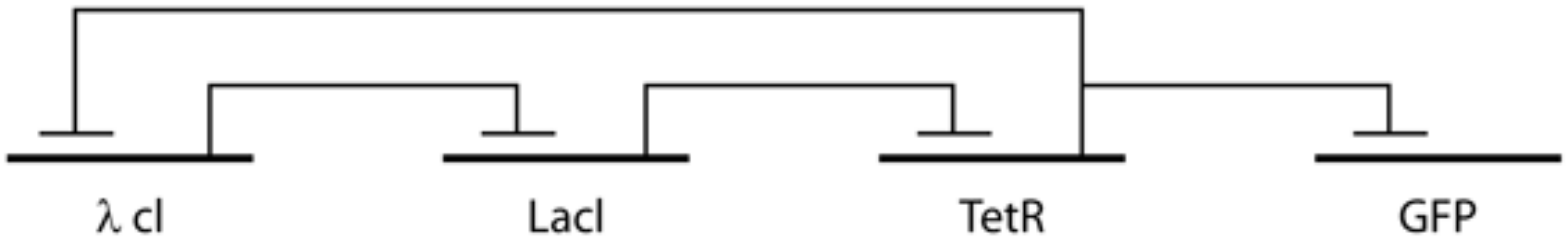


The Repressilator

What is a Repressilator?

- Synthetic genetic regulatory network reported in a paper by Elowitz and Leibler (2000).
- This network was designed from scratch to exhibit a stable oscillation which is reported via the expression of green fluorescent protein, and hence acts like an electrical oscillator system with fixed time periods.
- The network was implemented in E-coli bacteria using standard molecular biology methods and observations were performed that verify that the engineered colonies do indeed exhibit the desired oscillatory behavior.

Repressilator explained



- There are 3 genes.
- Whenever any one protein dominates over the others, it leads to its own repression – the dominant protein de-repress its own repressor, which then becomes dominant.
- Chain of 3 inhibitions.

Repressilator Mathematical Model

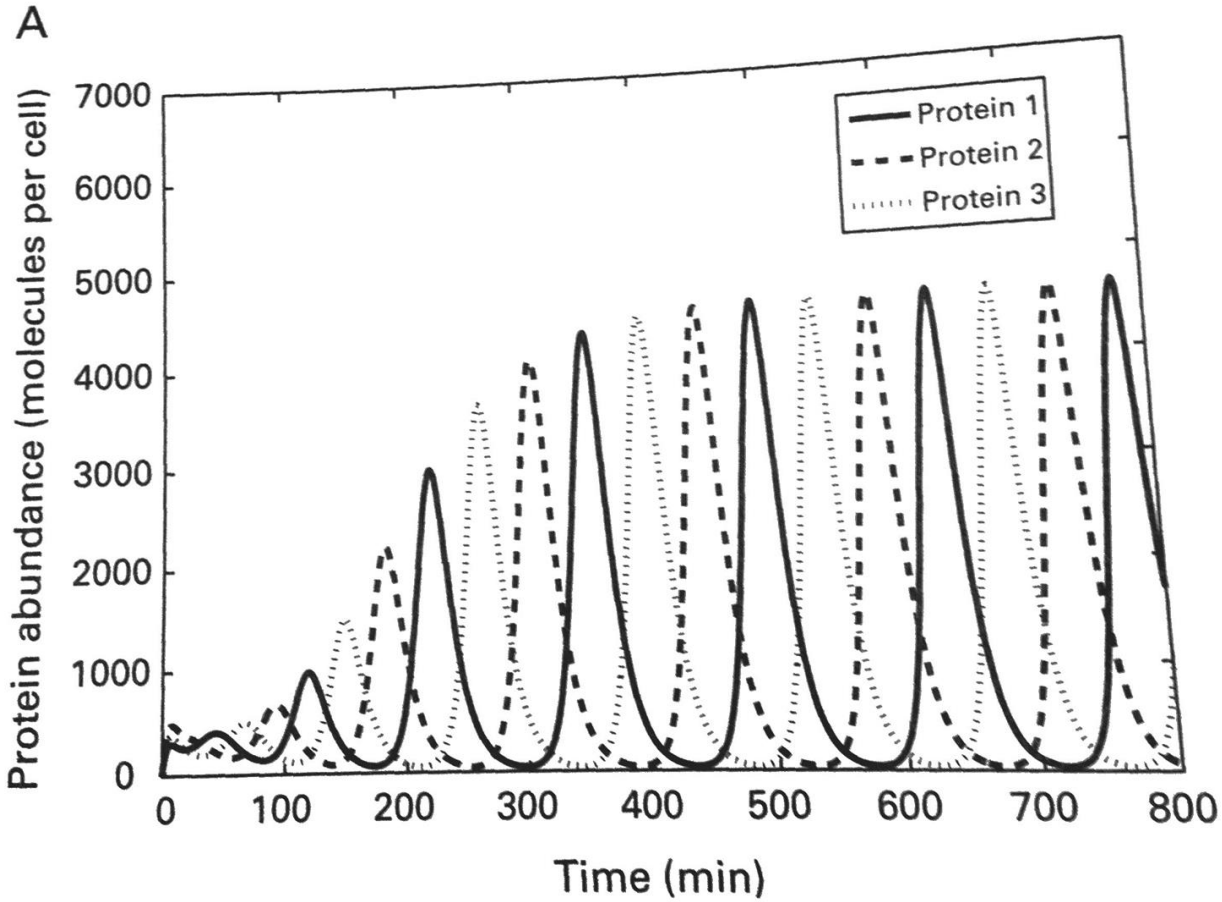
$$\frac{dm_1(t)}{dt} = \alpha_0 + \frac{\alpha}{1+(p_3(t))^n} - m_1(t) \qquad \frac{dp_1(t)}{dt} = \beta m_1(t) - \beta p_1(t)$$

$$\frac{dm_2(t)}{dt} = \alpha_0 + \frac{\alpha}{1+(p_1(t))^n} - m_2(t) \qquad \frac{dp_2(t)}{dt} = \beta m_2(t) - \beta p_2(t)$$

$$\frac{dm_3(t)}{dt} = \alpha_0 + \frac{\alpha}{1+(p_2(t))^n} - m_3(t) \qquad \frac{dp_3(t)}{dt} = \beta m_3(t) - \beta p_3(t)$$

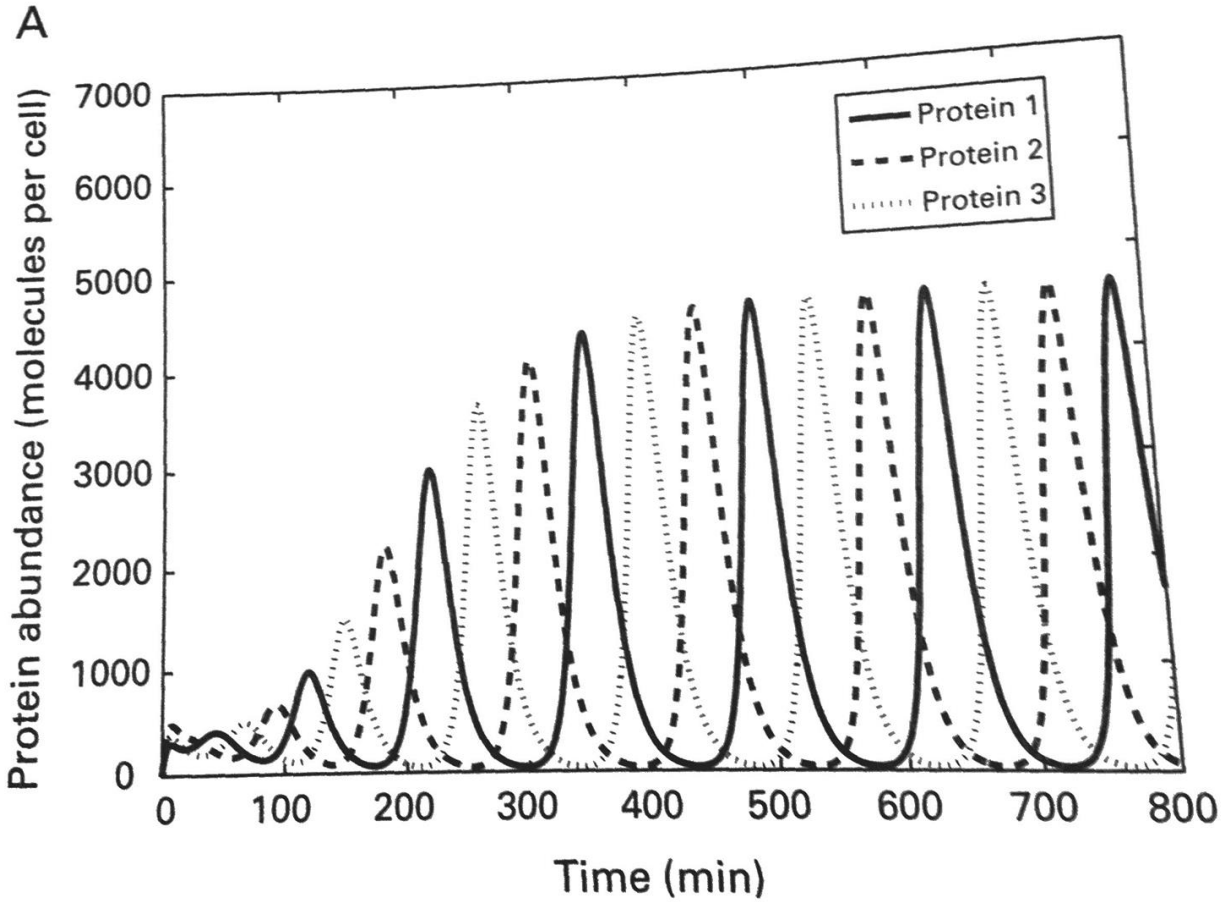
- There are 6 states – mRNA concentrations and protein concentrations
- Model include similar “leaky effects”, maximal expressions, protein degradation rates and Hill coefficient ←→ Time and magnitude scaling

Simulation results



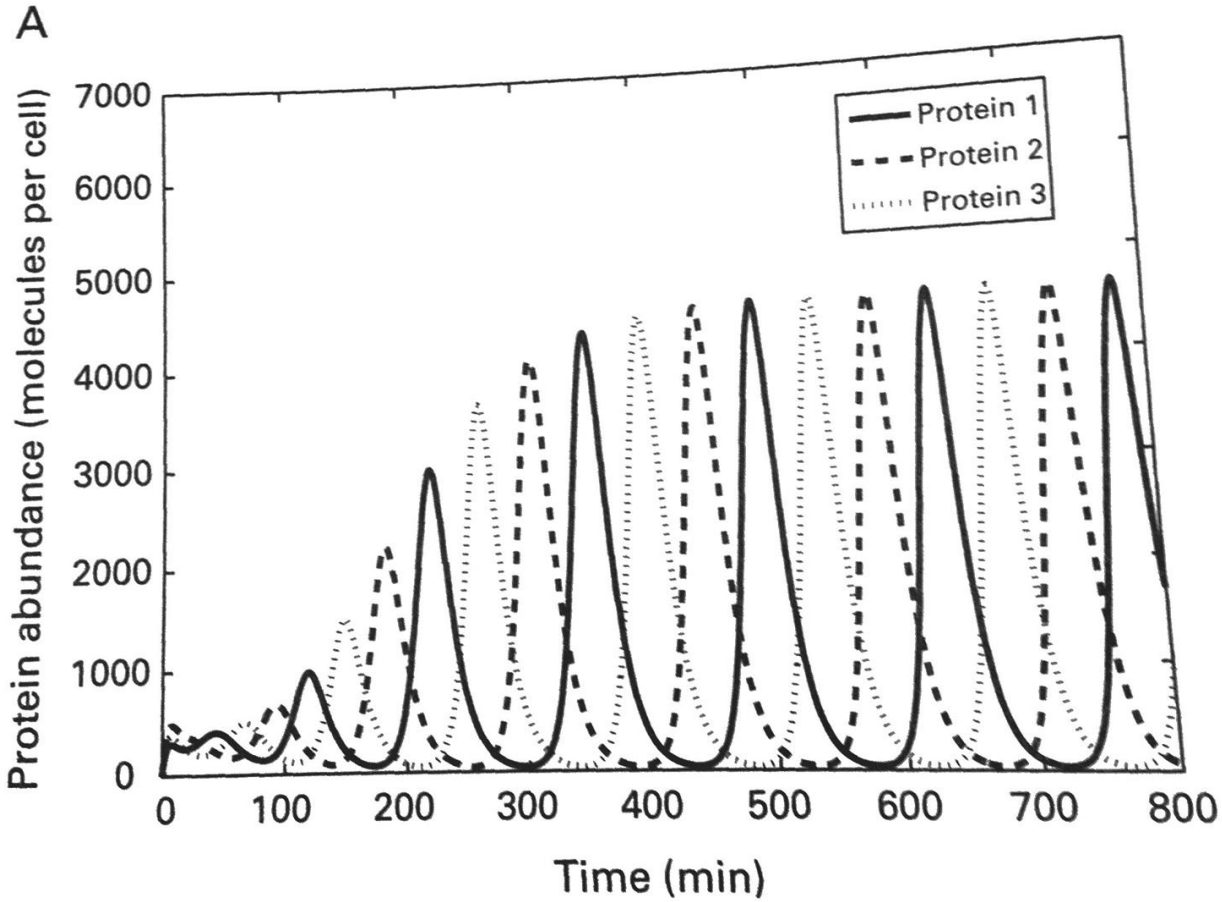
Parameters are $\alpha_0 = 0.03$ [molecules/cell/min], $\alpha = 298.2$, $\beta = 0.2$ [min^{-1}], n=2

Simulation results



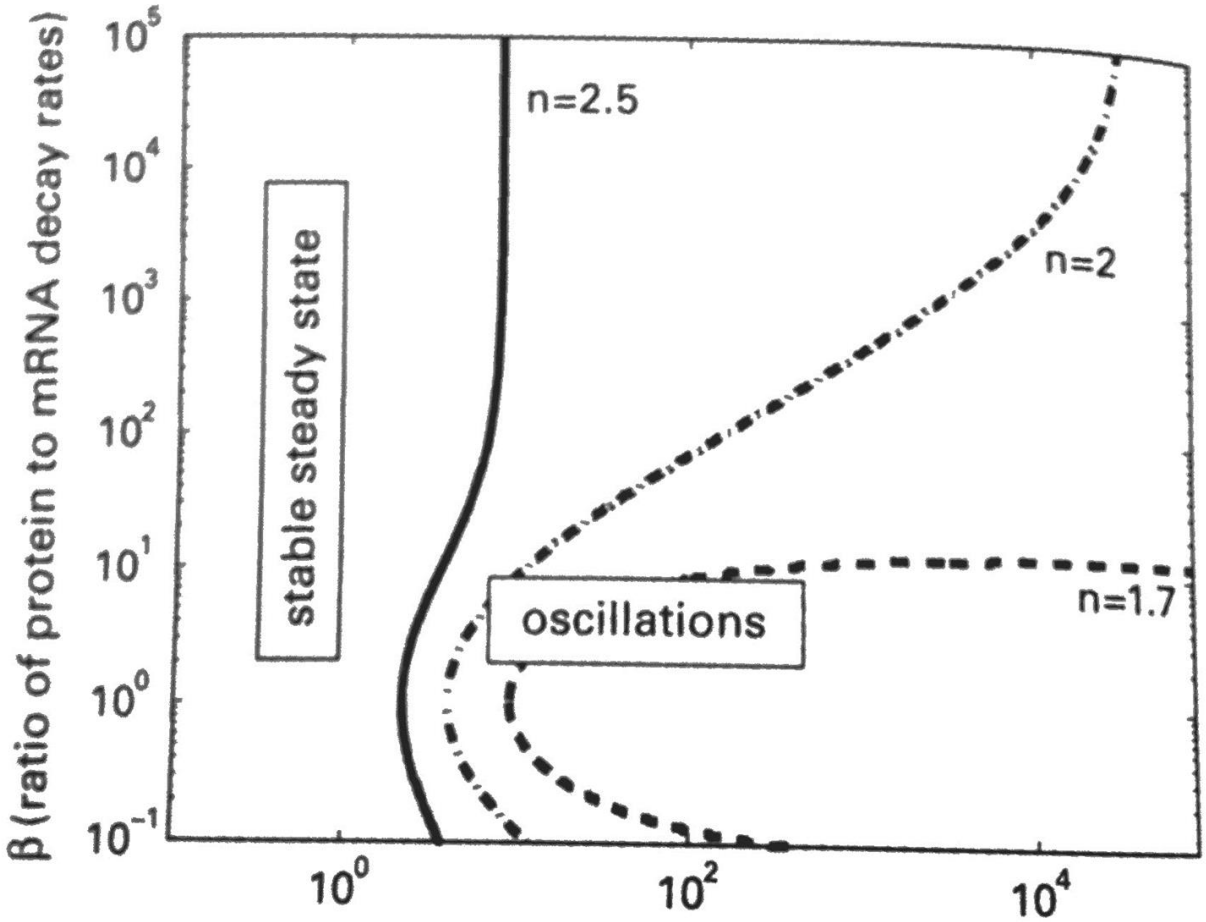
Magnitude scaling of 40 for protein concentrations; Time scaling of 1/0.3485 with respect to 2 minutes of half-life of mRNA

Simulation results



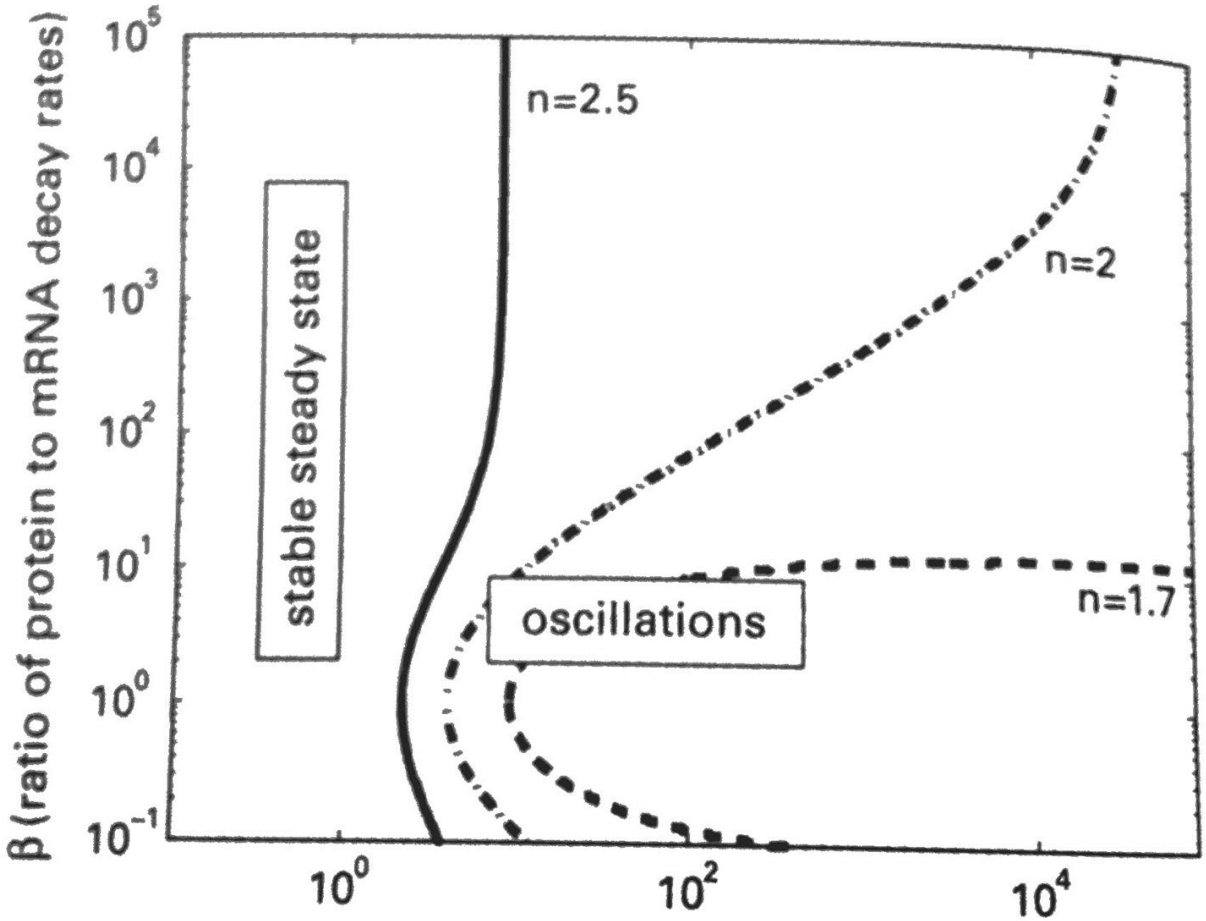
Due to symmetry every protein response have the same size and timing; Total period tuned to be 150 minutes

Parametric Study of the Repressilator



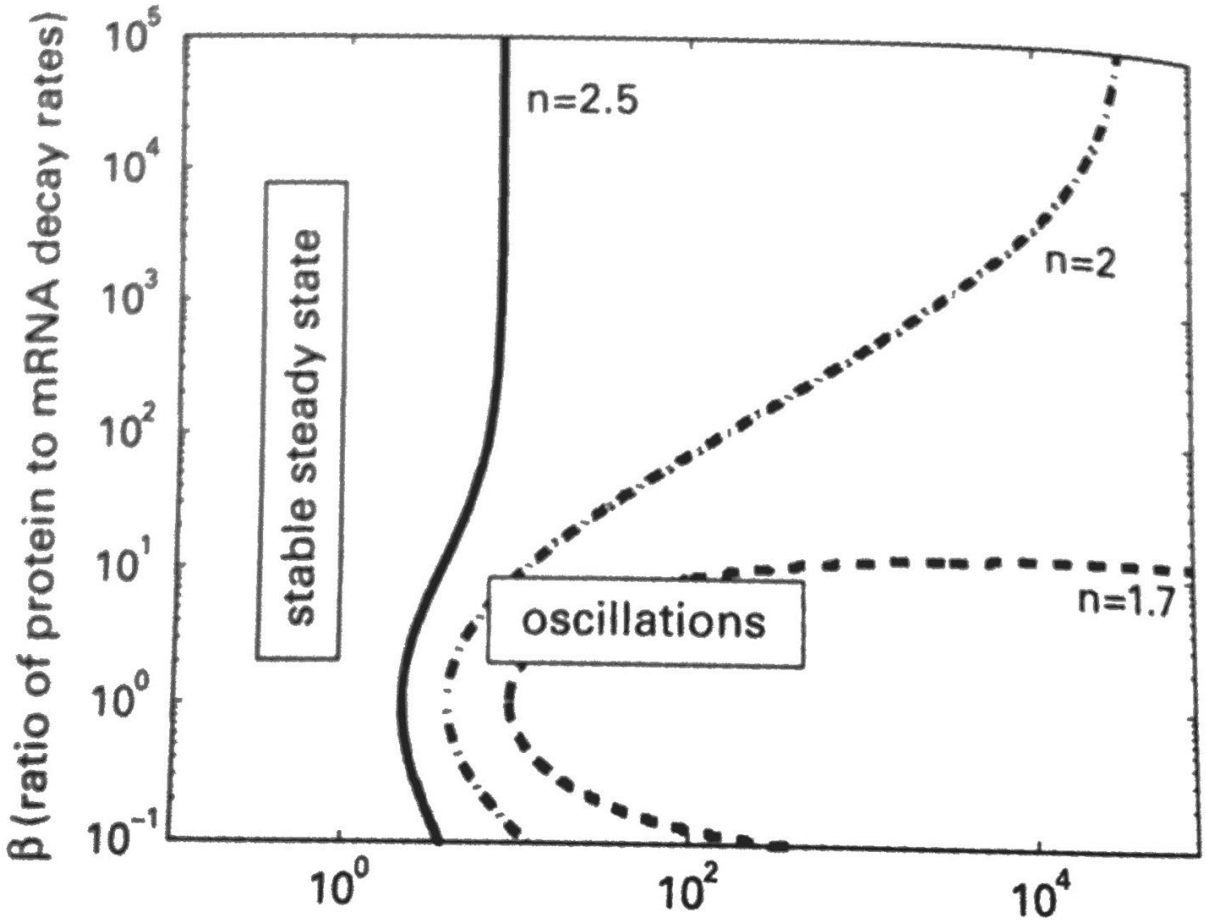
The X axis is α (the maximal expression rate).
Y axis is β (the ratio between protein decay rate and mRNA decay rate)

Parametric Study of the Repressilator



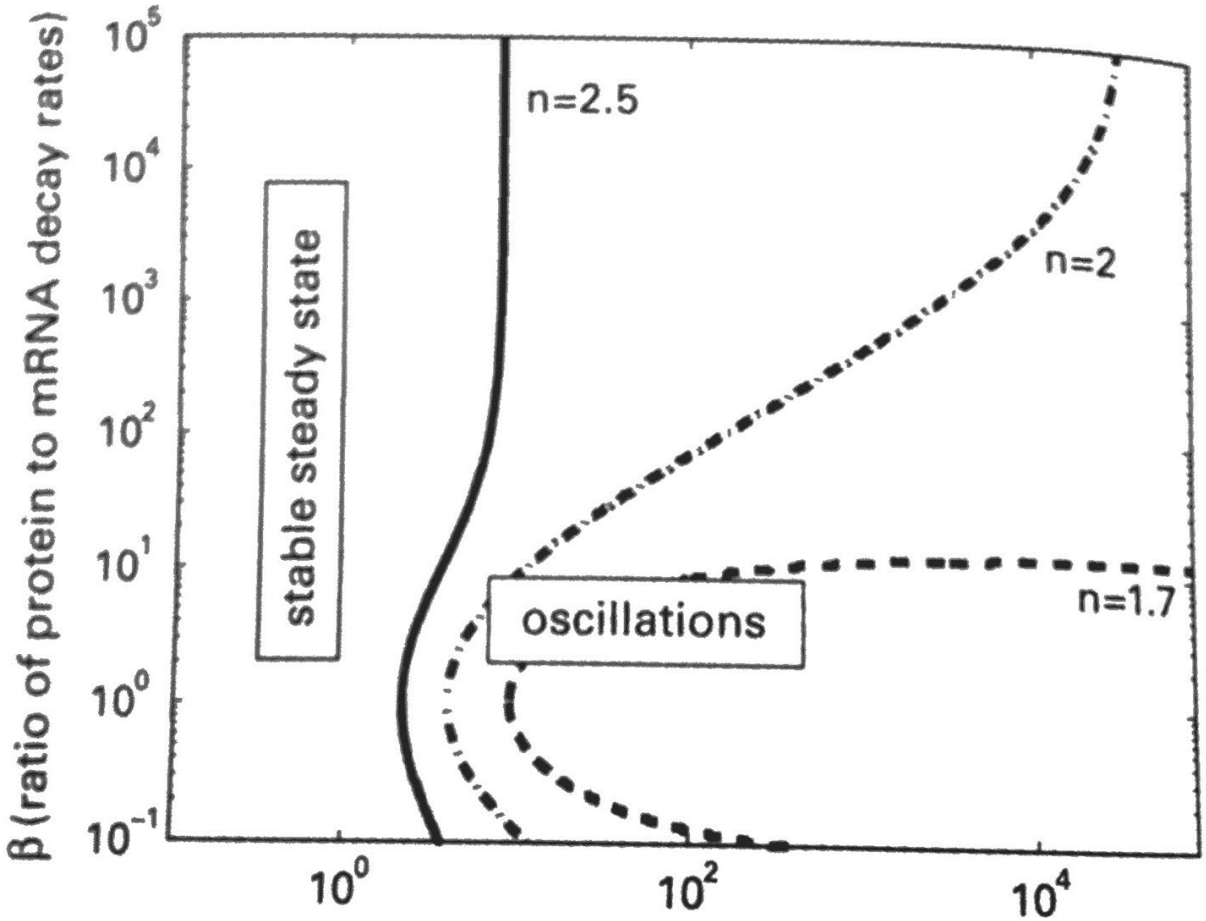
The larger n (repression nonlinearity) → the larger is the parameter space that creates oscillations.

Parametric Study of the Repressilator



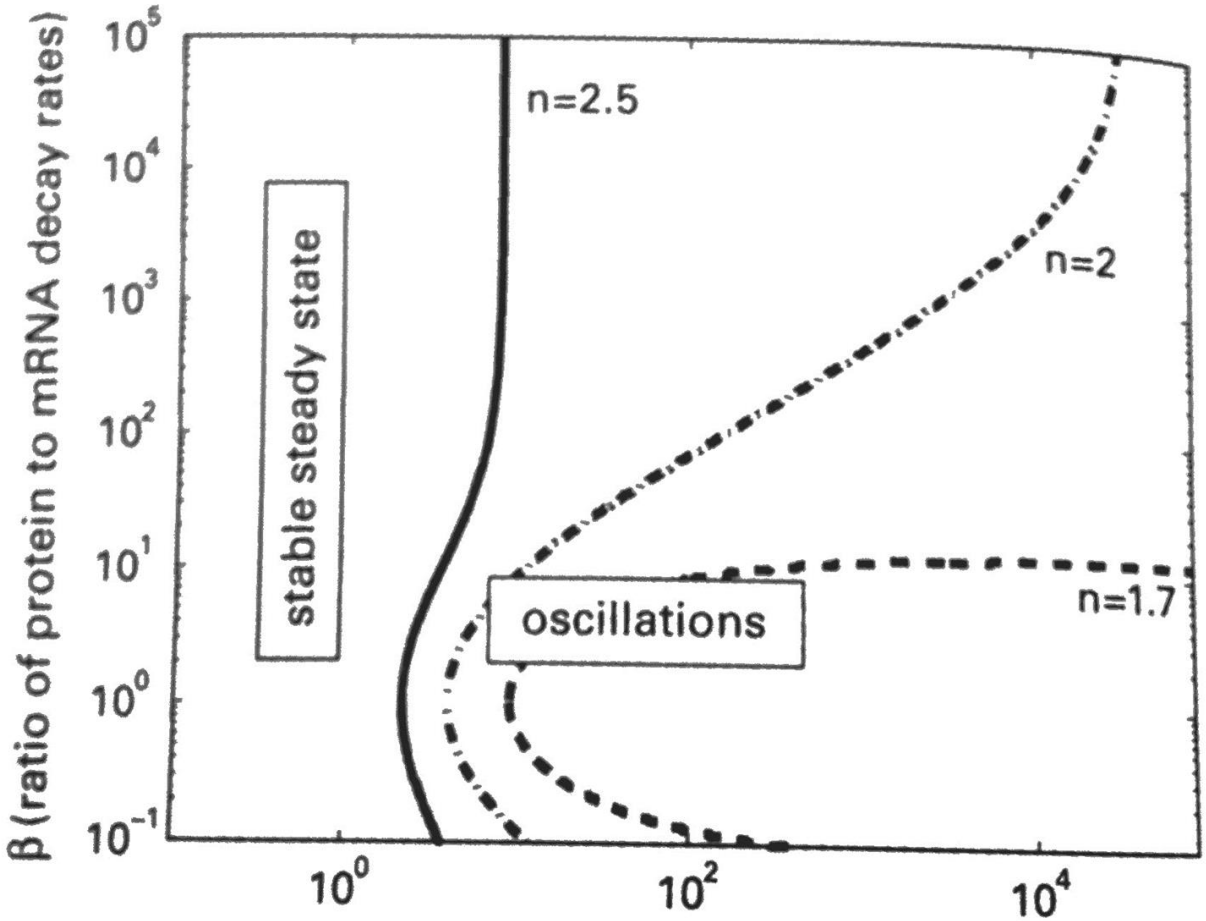
For given values of β and n → the larger the expression rate α the easier it is to create oscillations

Parametric Study of the Repressilator



For any values of β and n → If the expression rate α is too small, oscillations may not be possible

Parametric Study of the Repressilator



For given values of α and n → β should not be too large or too small in order to create oscillations – around 1 is best

More Simulations Observations

- Smaller values of the leakiness α_0 encourage oscillations.
- In the shown numerical model, if α_0 is increased from 0.03 to 2 the oscillations stop.
- For $\alpha_0 = 2$ the oscillations can be renewed if n is increased.
- For oscillations one needs an odd number of repressions (an idea taken from Ring Oscillators in Digital Logic)

Experiment Design (Elowitz and Leibler)

- Promoters chosen that were known to be cooperatively repressed (with high n values).
- Genes were selected to have high expression rates α.
- The leak coefficient was made very small by adding a “degradation tag” to the proteins in the network. It reduced their half-lives by factor of 15.

Experiment Design (Elowitz and Leibler)

- Well understood promoter-repressor pairs were selected:
  - LacI and TetR (from E. coli) – same as used in a switch called Collins' Switch.
  - Phage Lambda's cI.
- They were able to synchronize a population of E. coli cells hosting the network by exposing them to a pulse of IPTG (which inhibits LacI), demonstrating oscillations.

Experiment Design (Elowitz and Leibler)

- They were able to synchronize a population of E. coli cells hosting the network by exposing them to a pulse of IPTG (which inhibits LacI), demonstrating oscillations.
- IPTG is a non-metabolizable analogue of allolactose.
- Oscillations were demonstrated over 150 minutes, which interestingly is much larger than the doubling time of the cells (about 30 minutes) ←→ State of oscillations passed from mother to daughter cells after division.