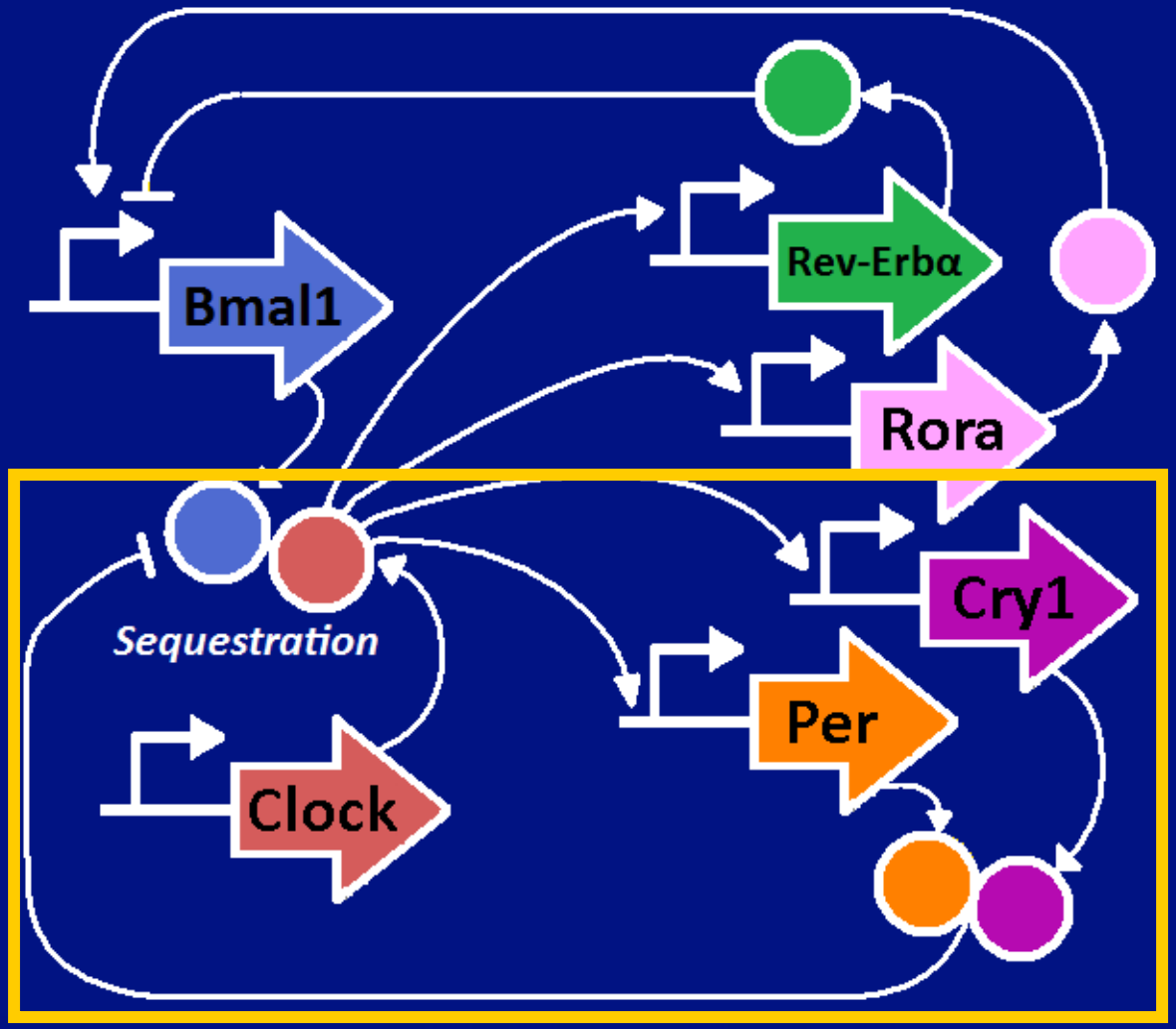
University of Michigan

iGEM 2008: *Beauty*... and the CLOCK

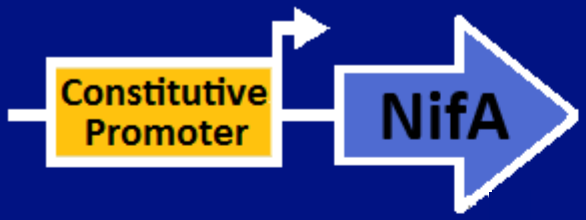
Background: Circadian Clocks

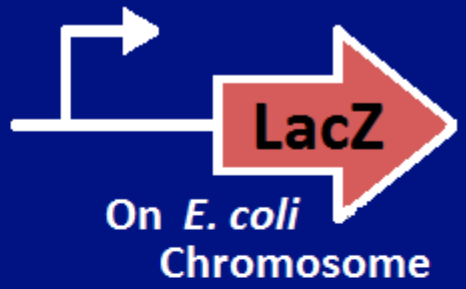
- Circadian rhythms represent natural oscillators
- Mammalian oscillators can be modeled with nonmammalian proteins
- Study of synthetic circadian clock aids understanding of natural circadian clocks
- Our synthetic clock focuses on sequestration step of mammalian clocks

Mammalian Circadian Clock



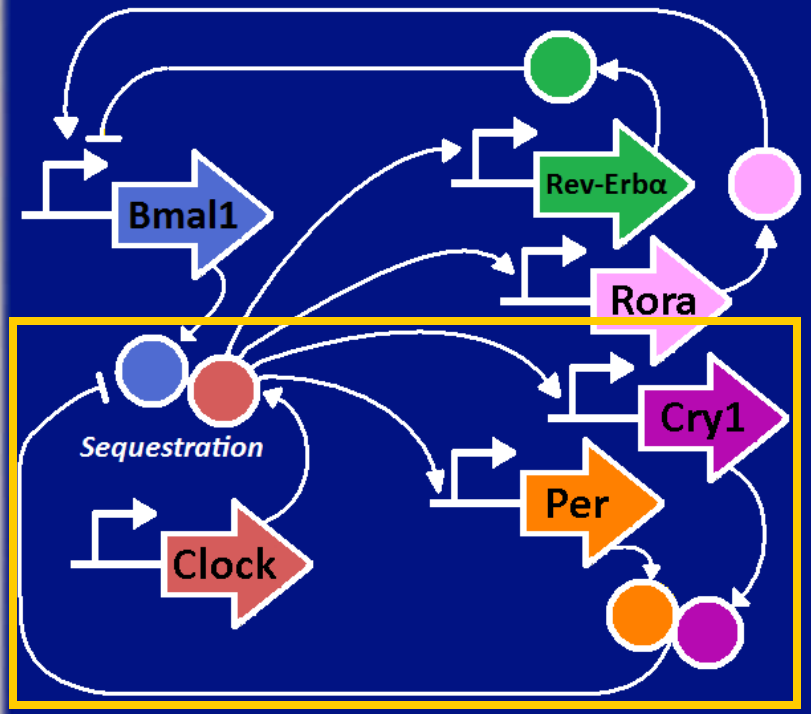
Project Description: The Sequestillator



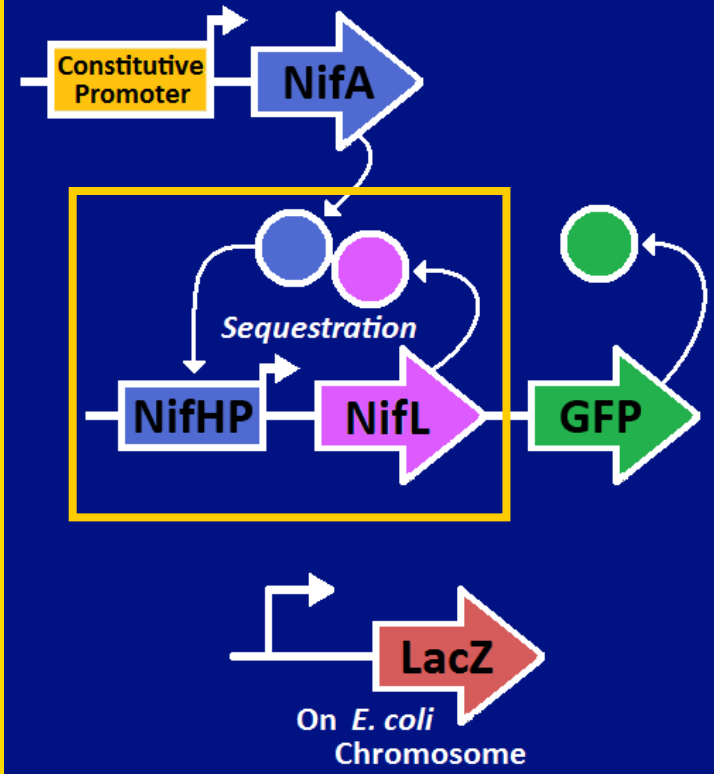


Comparison of Circadian Clocks

Natural Circadian Clock

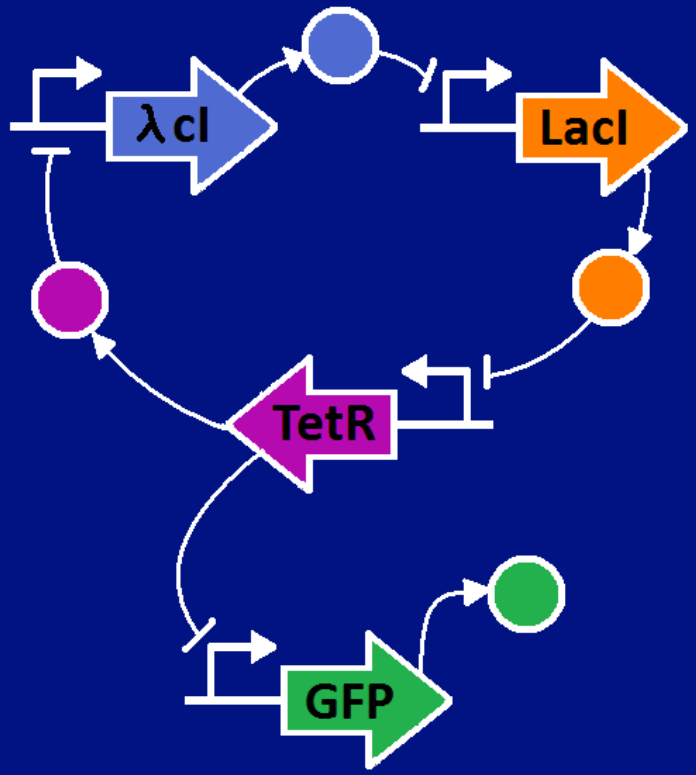


The Sequestillator



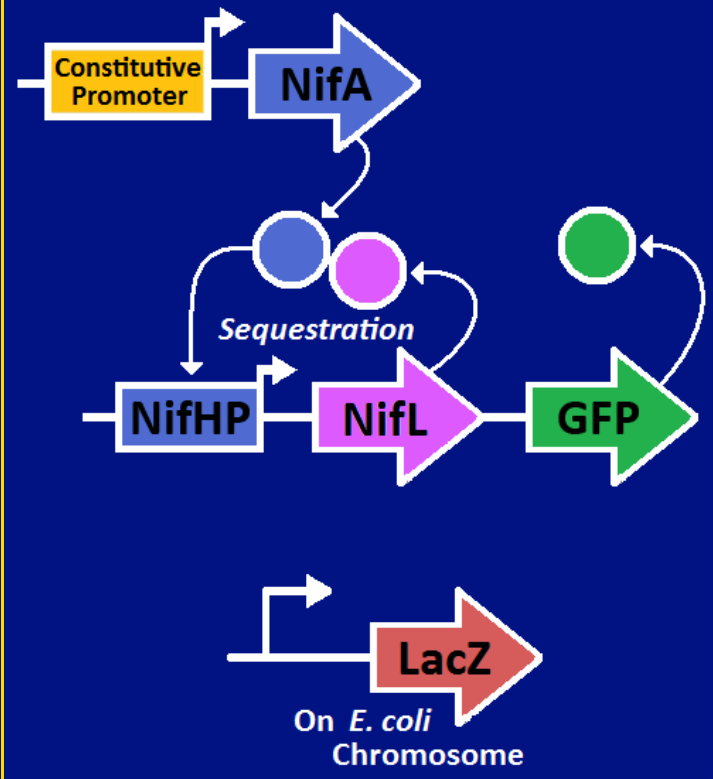
Previous Synthetic Clocks

The Repressilator



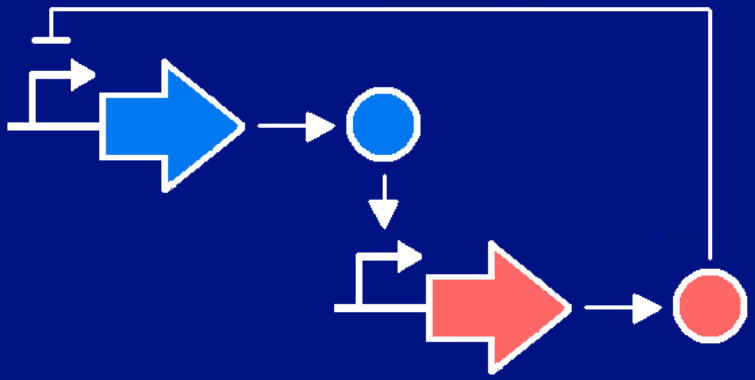
(Elowitz and Leibler, *Nature*, 2000)

The Sequestillator

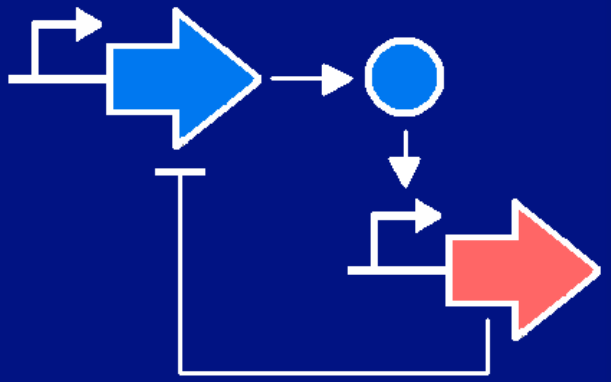


Modeling

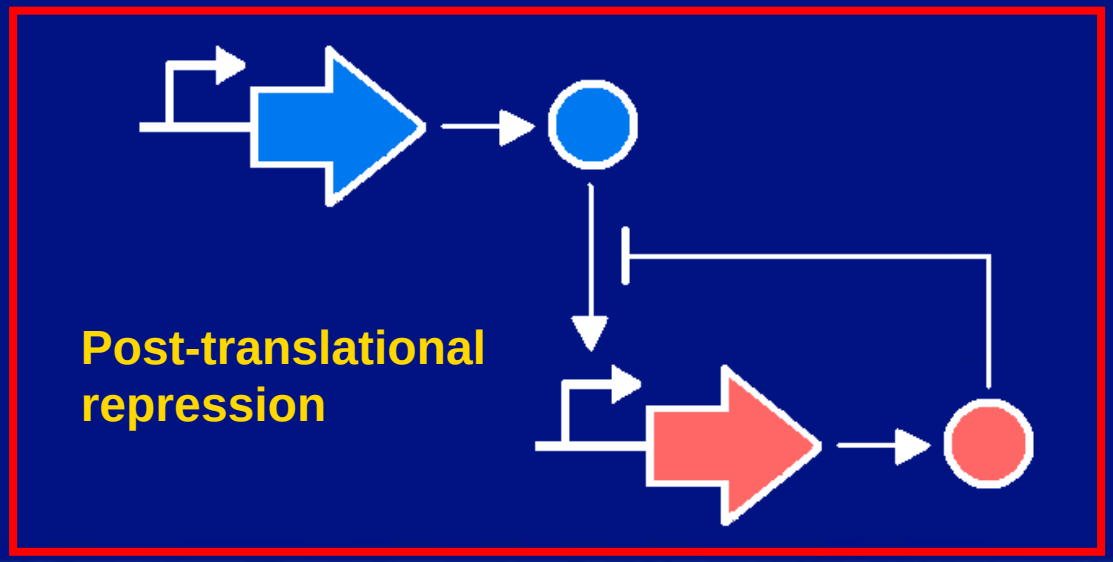
Focus of Modeling



Pre-transcriptional repression



Pre- translational repression



Will this thing even work?

- In fancier terms: How robust?
  - Criteria for robustness?
  - A possible “value”: divide the number of parameter sets that give oscillations over the total number of feasible sets of parameters
    - Colloquially, we call this the Numerical Indication Noting Found Activity (NINFA) Index.

Ninfa Index Calculator ("Indexilator")

Run ODE simulation at time interval far from start of run (time = 1000, 2000, etc.)

↓

Fast Fourier Transform of solution trajectory; Transform to get Power Spectral Density (PSD)

↓

Search for two symmetric peaks in the PSD, by searching for two equal maxima in the plot

↓

Low pass filter: eliminate "weak" oscillations by requiring PSD maxima to be above small threshold

↓

Compare amplitudes at the beginnning of the time interval and compare to that at the end of the interval - eliminate if not within 10% of each other (select against damped oscillations)

Repeat for multiple parameter sets

Models

Model 1: Modeling of the Sequestillator

$$\frac{d[m]}{dt} = \frac{t_{\max}}{2A_t}[-(L_B - A_t + K_d) + \sqrt{(L_B - A_t + K_d)^2 + 4A_t K_d}] - d_m m$$

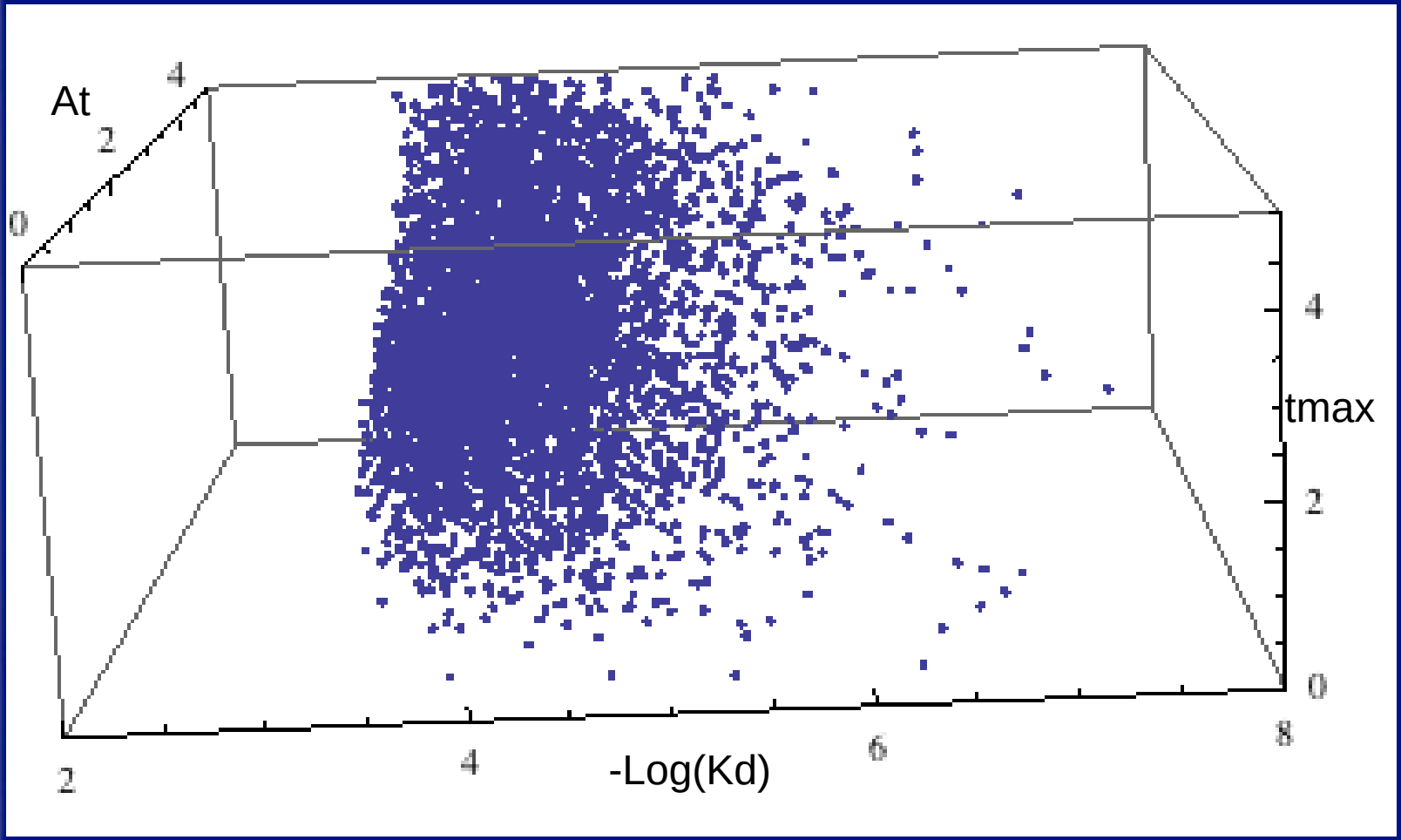
$$\frac{d[L_A]}{dt} = t_l m - (d_{L_A} + r) L_A$$

$$\frac{d[L_B]}{dt} = r L_A - d_{L_B} L_B$$

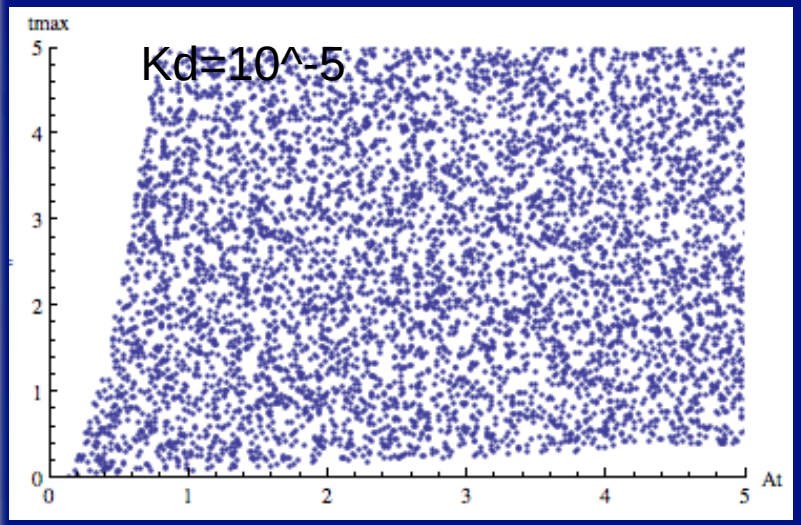
- Kd= NifL/NifA dissociation constant
- At= amount of NifA in system
- Any d= degradation rate
- tmax, tl = max.transcription/translation rate
- r= rate of activation of NifL

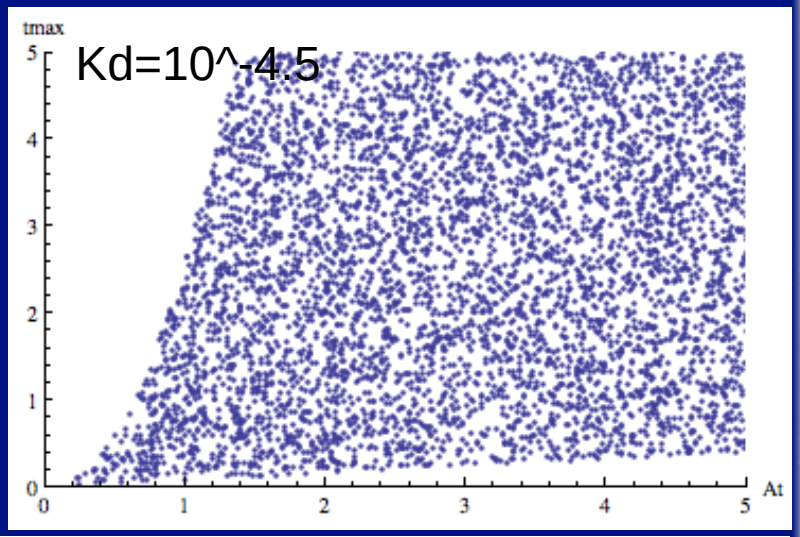
- Complex mRNA production function derives from rapid steady state assumptions
- Oscillation Requirements: tight binding of NifL/NifA; one-to-one titration of species

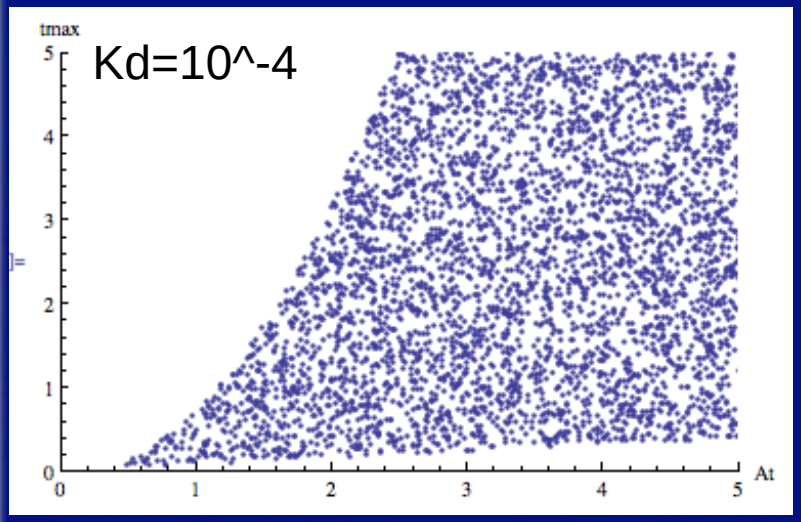
Model 1: Cloud

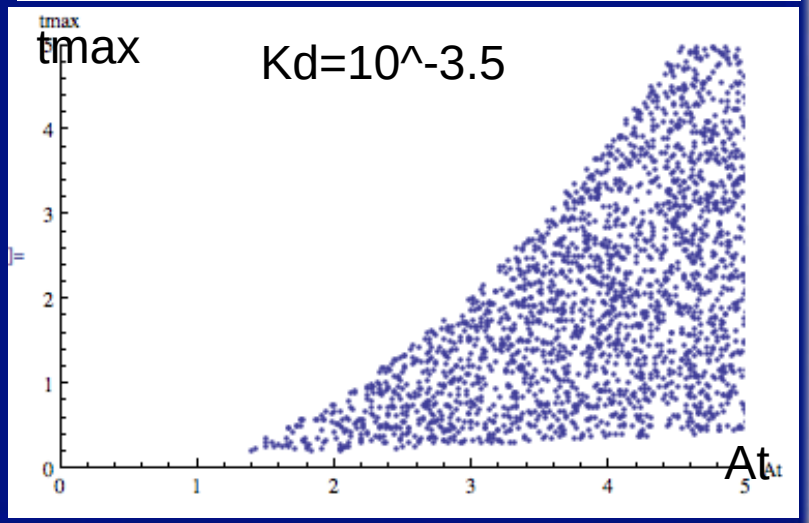


Model 1: Slices

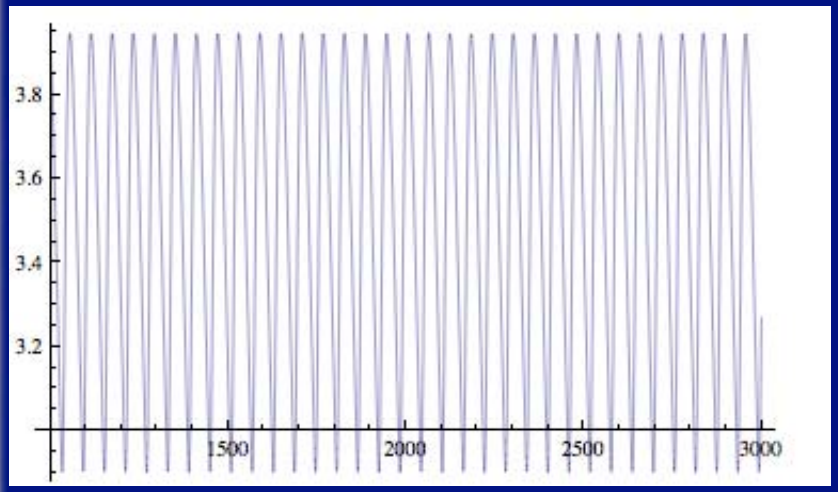


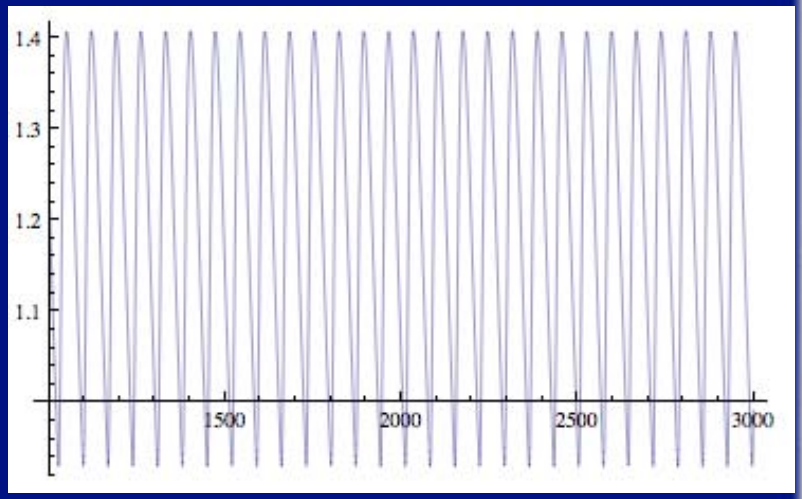


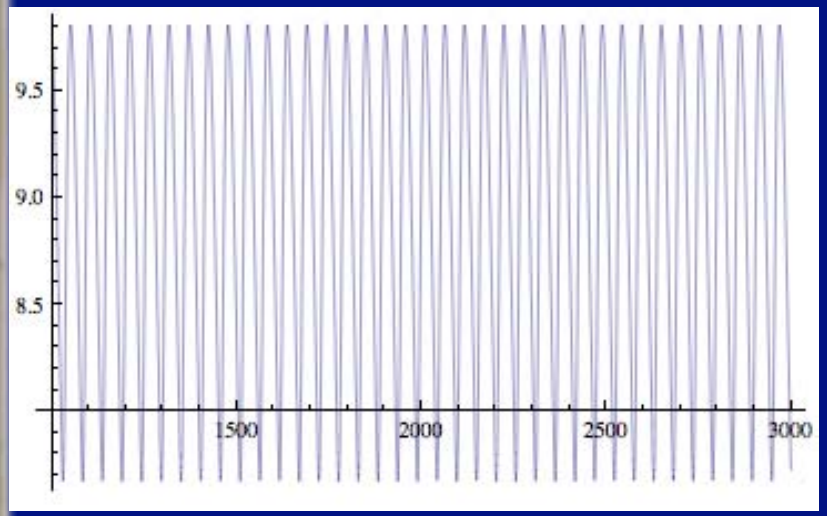


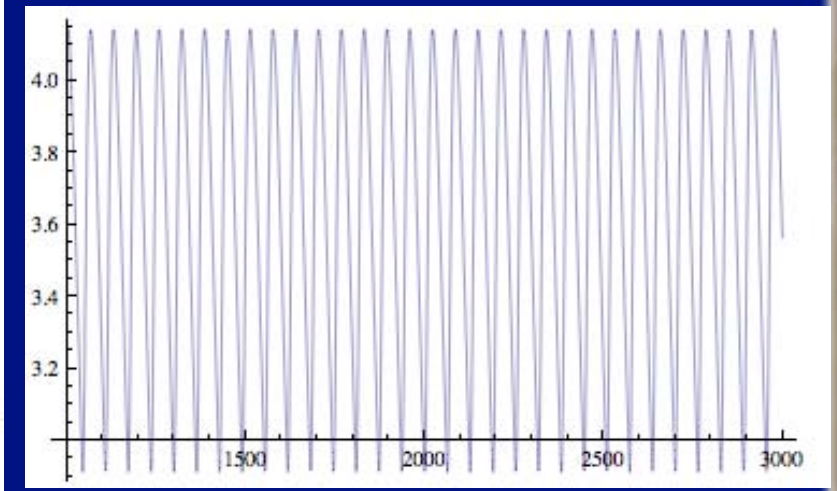


Model 1: Trajectories

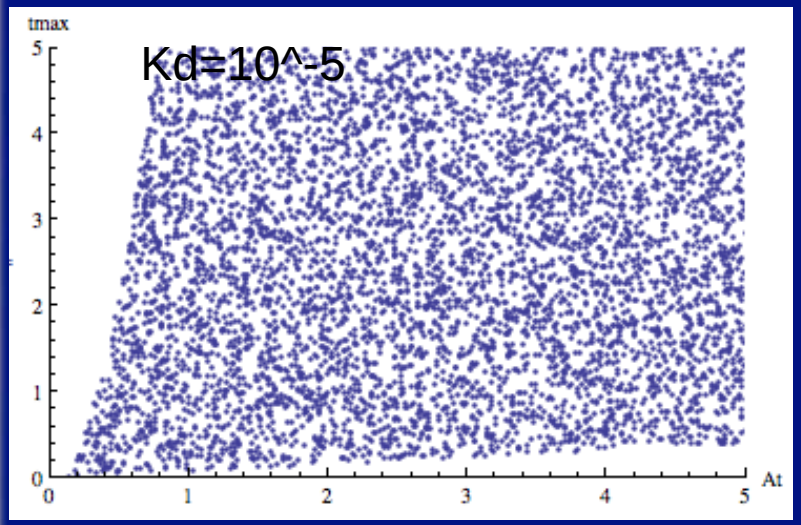


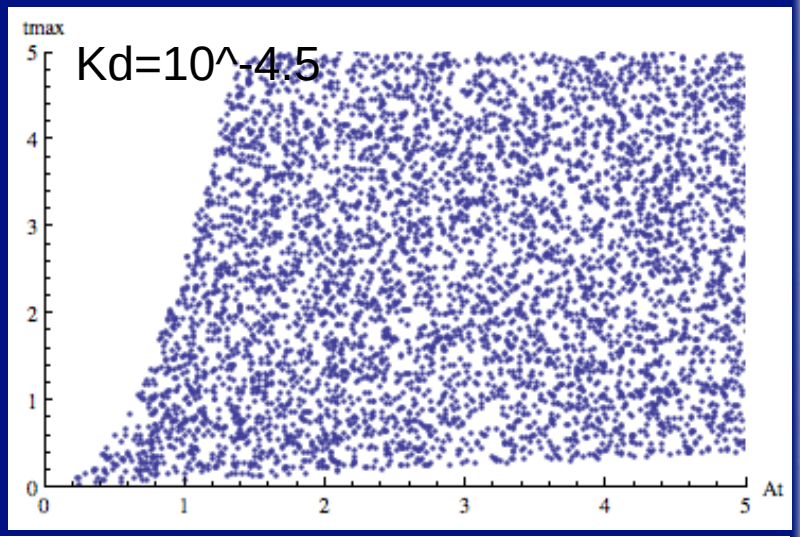


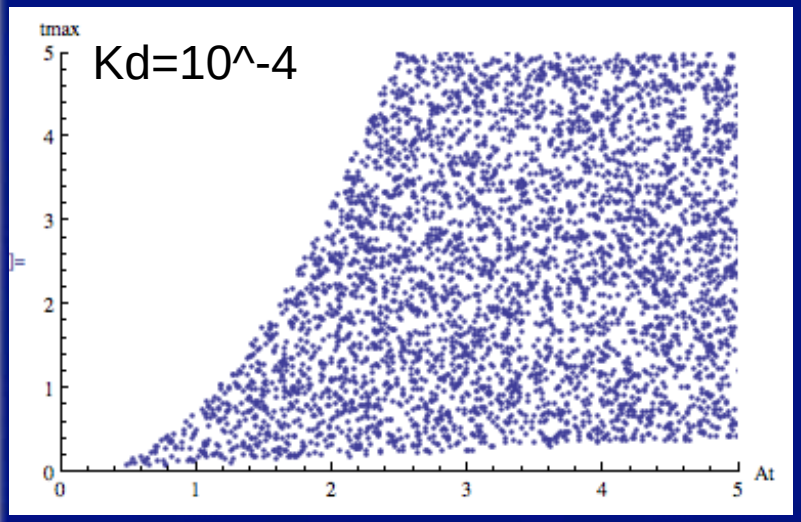


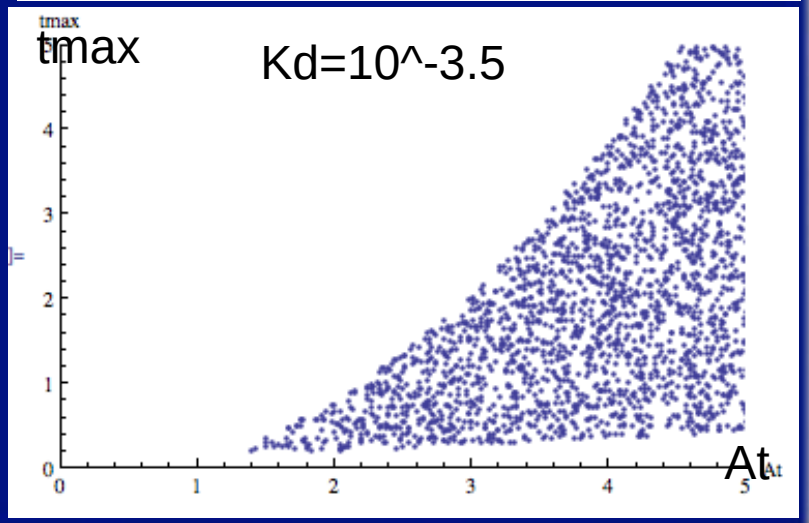


Model 1: Slices





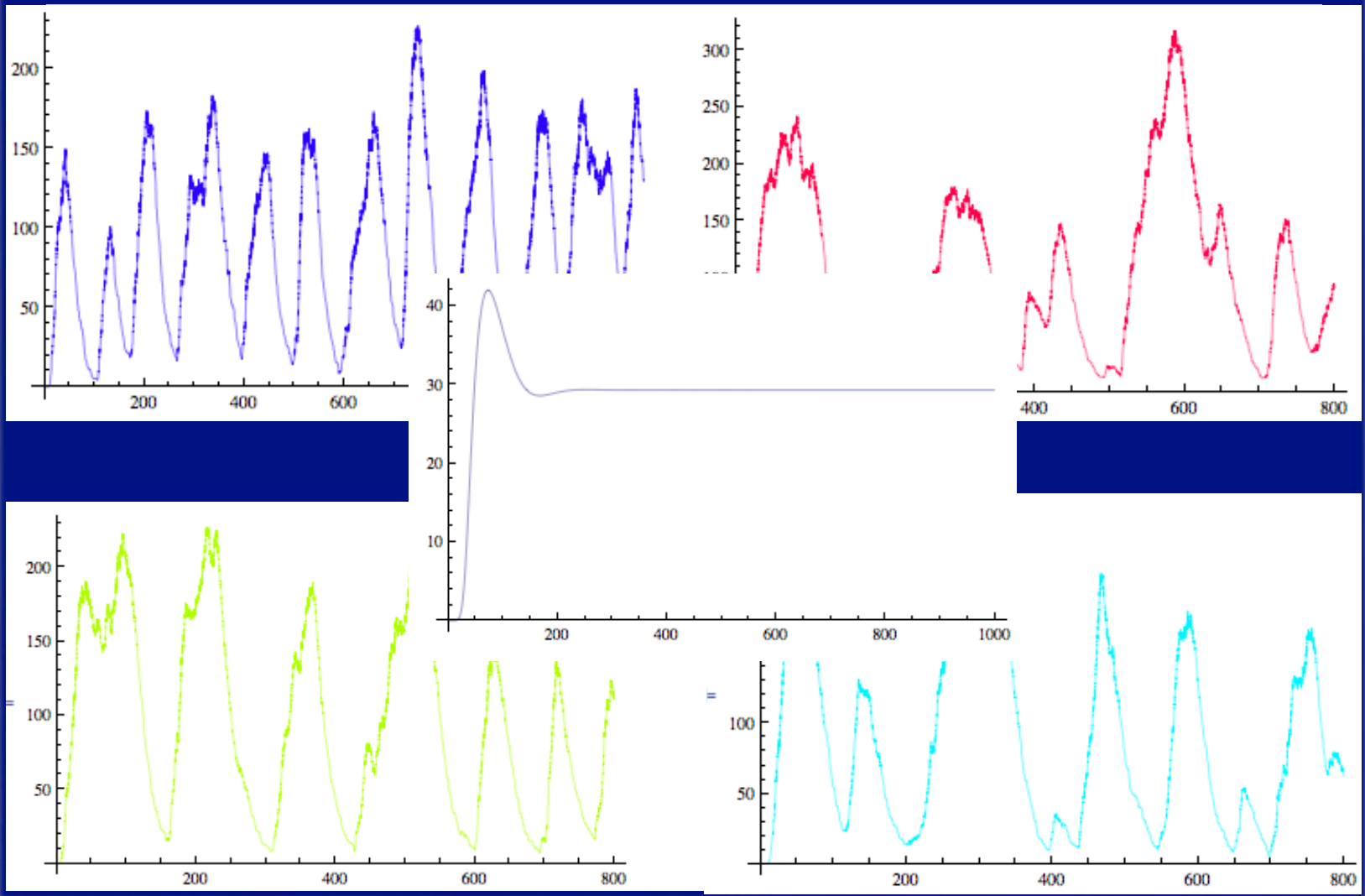




Ninfa Index Searches on Model 1

Parameter Search Ranges	Ninfa Index
r –between 0 and 1 tmax –between 0 and 5 Kd –between 0 and .0001 At – between 0 and 5	**0.542725** (40000 Simulations)
r –between 0 and 1 tmax –between 0 and 5 Kd –between 0 and .1 At –between 0 and 5	**0.00108585446** (152875 Simulations)

Stochastic Results



Landing Pads

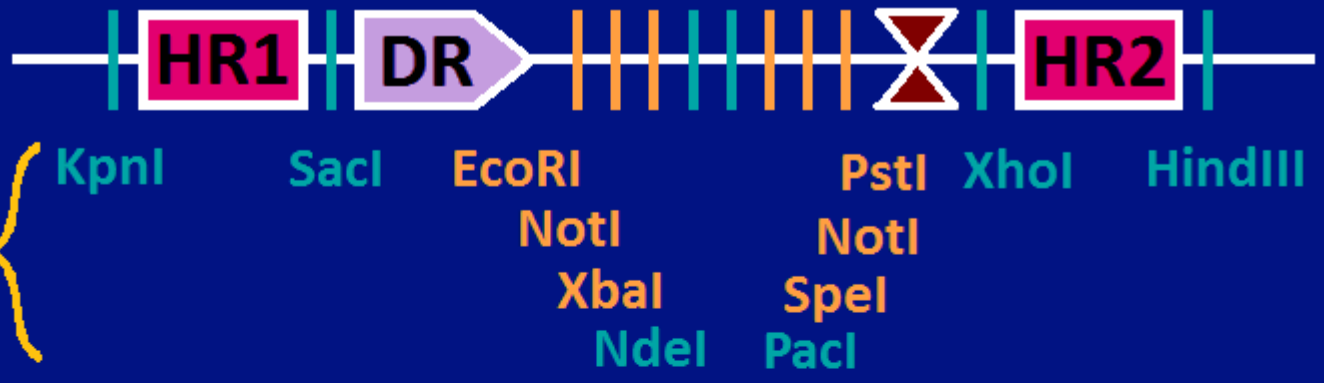
Landing Pads

iGEM 2007 Project: BioBrick Landing Pad

- Landing Pads: Aid in insertion of constructs onto the chromosome

- Goals of BioBrick Landing Pad:
  - BioBrick Compatibility
  - Allow easy phenotypic screening
  - Limit Noise

Landing Pads

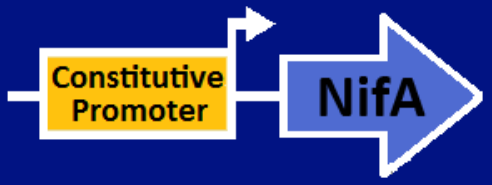


Key:

- Homologous Regions
- Drug Resistance Gene
- Bar Code Sequence
- Building Restriction Sites
- BioBrick Restriction Sites

Landing Pads

Sequestillator & Landing Pads:

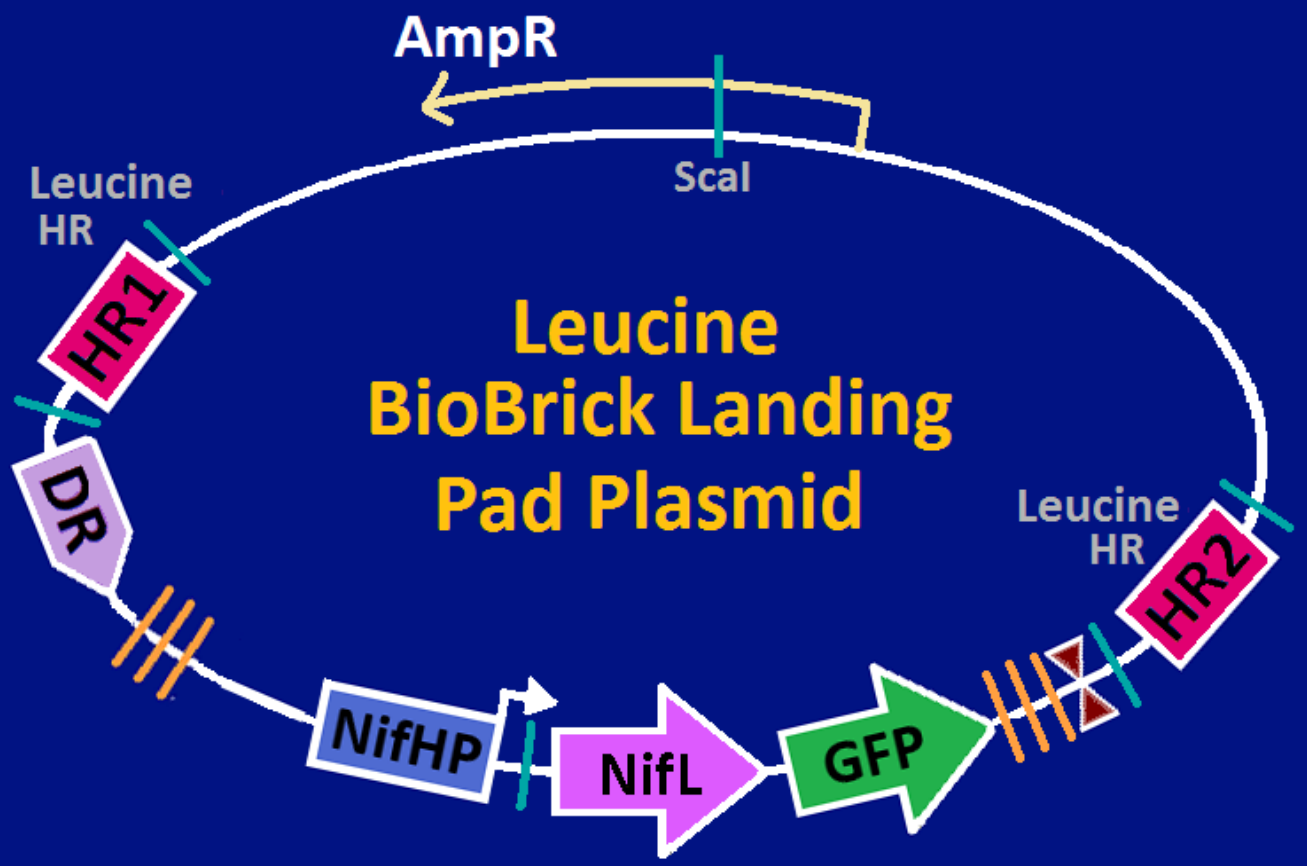
Operon	Topology	Landing Pad
Activator Operon		Arabinose Landing Pad*
Repressor Operon		Leucine Landing Pad**

* Created for iGEM 2007 by Steve Selinsky, Khalid Miri and Alyssa Delke

** Created by former Ninfa lab member Dr. Dong Eun Chang

Landing Pads

Repressor Operon:



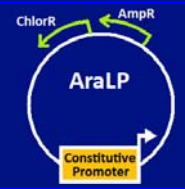
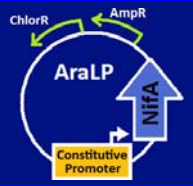
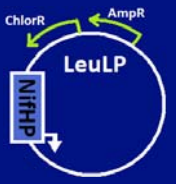
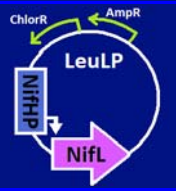
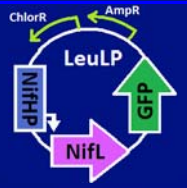
Fabrication

Fabrication of the Sequestillator

Fabrication of the Sequestillator

BioBrick General Promoter

Fabrication of the Sequestillator

Operon	Description of Part	Completed?
Activator Operon	CP in AraLP 	In Progress
	NifA & CP in AraLP 	In Progress
Repressor Operon	NifHP in LeuLP 	Completed
	NifL & NifHP in LeuLP 	Completed
	GFP, NifL & NifHP in LeuLP 	In Progress

Registry Parts

Part Name	Description of Part	Registry Number
pCLOCK1	NifHP in Leucine LP	Bba_K138000
pCLOCK2	NifL & NifHP in Leucine LP	Bba_K138001
pAraLP	Arabinose Landing Pad	Bba_K138002

Conclusion

- Novel framework for replicating the mammalian clock in E. coli using nitrogen fixation genes, landing pads, and NINFA index software
- Our clock will aid future research
- Landing pads and fabrications are available to the public in the BioBrick registry
- NINFA index software is available on our team wiki page

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Questions?

Model 2: More Detailed

- Account for mRNA, dimerization

$$\frac{d[m_L]}{dt} = t_{\max}\frac{{A_{2i}}^m}{k^m + {A_{2i}}^m} - d_m m_L$$

$$\frac{d[L]}{dt} = t_l\, m_L - k_f L^2 + 2k_r L_2 - d_L L$$

$$\frac{d[L_2]}{dt} = .5k_f L^2 - (k_B A_{2i} - k_{B2} AL + k_r + d_{L_2})L_2$$

$$\frac{d[A]}{dt} = a - g(A, A_2)$$

$$\frac{d[A_{2i}]}{dt} = F_i(A_{2(i-1)}, A_{2i}, A_{2(i+1)}, AL)$$

Functions g and f encapsulate all of the dim*erization and binding reactions*

Trial	Parameter Search Ranges	Ninfa Indices
1	d's,k, kr: Randomized 0 to 1 tmax, tl, a: Randomized 0 to 5 kf: Randomized 1 to 100 kB: Randomized .5 to 1 kB2: Randomized 0 to .5	.3022 (16480 simulations)
2	Same as Trial 1, except: kf: Randomized 1 to 10	.08877 (16480 simulations)
3	Same as Trial 2, except: a: Randomized 0 to 1	.1505 (16480 simulations)
4	Same as Trial 2, except: a: Randomized 0 to 15	.03568 (16480 simulations)
5	Same as Trial 2, except: a, tmax: Randomized 0 to 15	.0882 (20000 simulations)

Although smaller indices than in Model 1 , system still has chance of oscillations!