Multistability in the lactose utilization network of *Escherichia coli*

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Purpose of paper
Biological background
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Measuring network parameters
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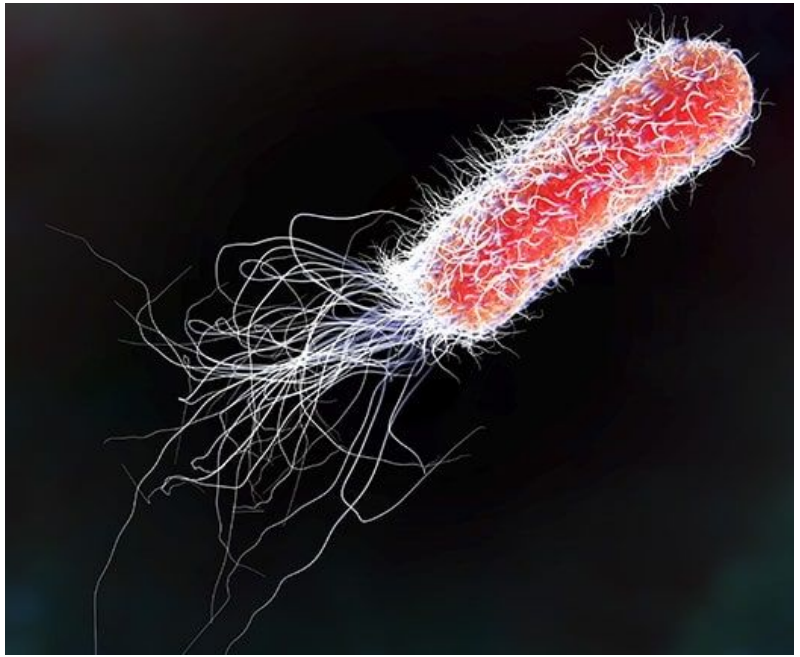


Source: BioCote

Motivation

- Recreate and verify mathematical results from “Multistability in the lactose utilization network of *Escherichia coli*” by Ozbudak et al.
  - Interest in applying mathematics to biology
  - Intersectional knowledge

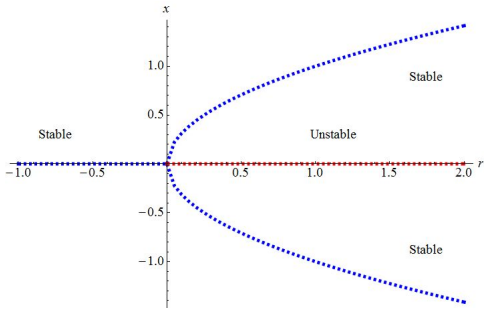
- Generate artificial data that mimics real-time data

- Apply mathematical model to other systems
  - Solid/liquid/gas phase diagrams
  - Compare - similarities and differences

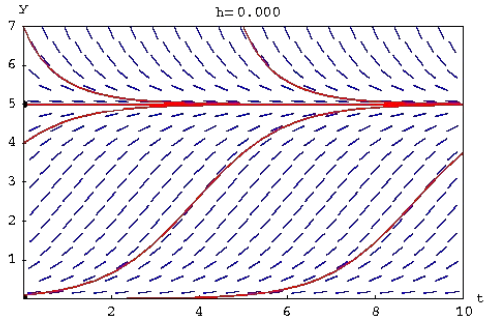
Multistability of (general) systems

$$\frac{dx}{dt} = rx - x^3$$

- Multiple internal states in response to single set of external outputs
- Biological “switches”
  - Essential for variety of processes
- Positive feedback loops responsible for multistability of systems
  - Loops do not guarantee multistability
- Phase diagrams
  - Internal states as external parameters vary
  - Determine requirements for switch within a system



Bifurcation diagram



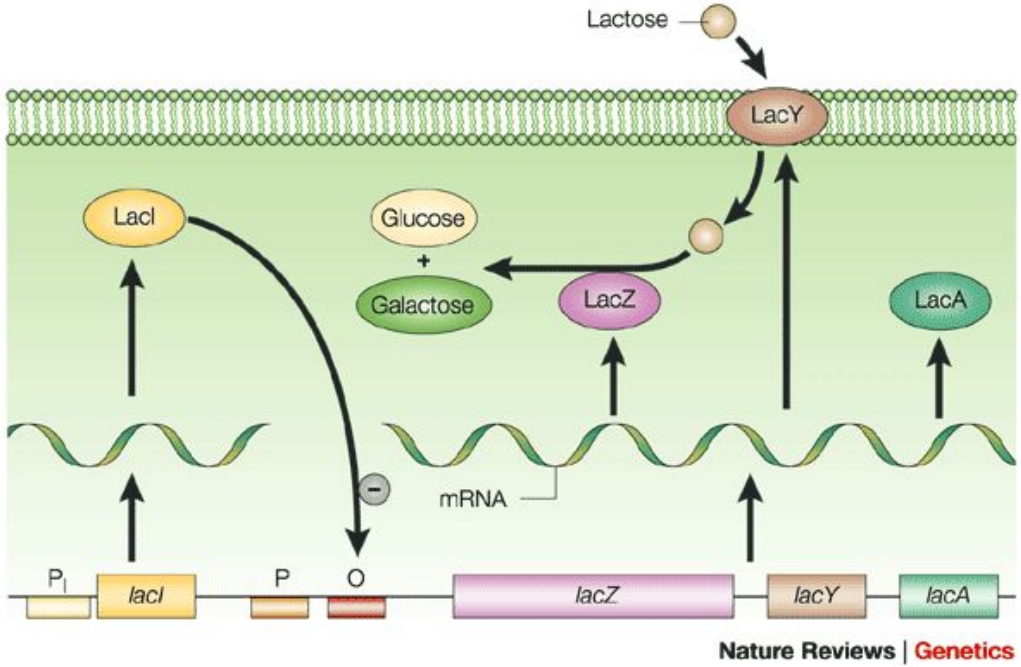
Varying parameters

Purpose

- Present phase diagram of lactose utilization network of *Escherichia coli*
  - BISTABLE
- Quantitatively investigate processes using phase diagram and mathematical model of network
  - Sugar uptake
  - Transcriptional regulation
- Show that hysteretic response of wild-type system can be converted to ultrasensitive graded response

Some biological background

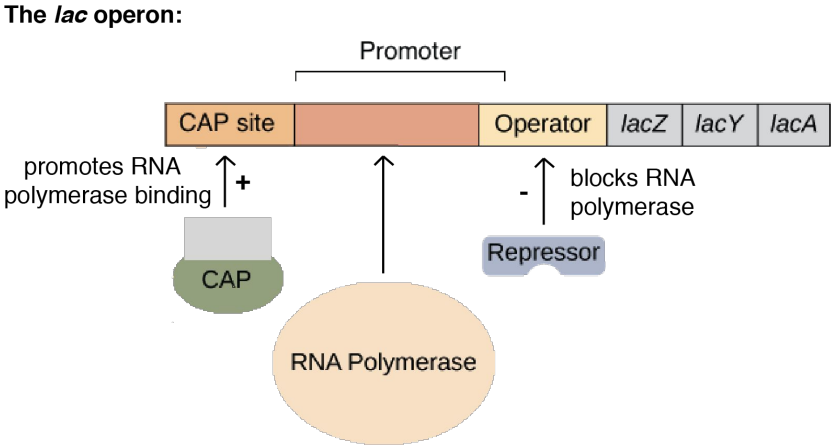
- *lac* operon
  - Three metabolic genes: *lacZ, lacY, lacA*
  - Genes required for uptake and metabolism of lactose
- Two transcriptional regulators
  - Repressor (LacI) turns off lactose metabolism
    - Inducers (TMG) inhibit repression
  - Activator (cyclic AMP receptor protein, CRP) triggers lactose metabolism



Source: Nature Education

More on the *lac* operon...

- Glucose present:
  - Low cAMP
  - Repressor binds to operator and blocks RNA polymerase
    - Repressor coded by LacI gene
  - Less transcription of *lac* operon
- No glucose present:
  - High cAMP
  - cAMP binds to CAP (an activator of transcription)
  - Allolactose binds to repressor to remove from operator
  - Lots of transcription of the *lac* operon to break down lactose



Source: Khan Academy

SO WHAT?

- Presence of TMG inhibits repression by LacI
- TMG and glucose affect the inhibitor and activator of *lac* expression independently

cAMP levels unaffected by TMG uptake, but affected by levels of glucose

- Uptake of TMG induces synthesis of lactose permease (LacY, coded by *LacY*), which promotes further TMG uptake and facilitates uptake of lactose
  - POSITIVE FEEDBACK LOOP → potential for bistability
- Require cells with well-defined initial states (not induced or fully induced) - response of the bistable system depends on its history (hysteresis!)

Methods

Vary 2 external inputs: extracellular concentrations of glucose and TMG

- Measure the levels of 2 fluorescent reporter proteins:
  - GFP - found at the lac promoter
  - HcRed - found at the gat promoter; direct measure of CRP-cAMP levels
- Note:
  - TMG inhibits the inhibitor of GFP, therefore activating GFP
  - Glucose inhibits the production of GFP & HcRed

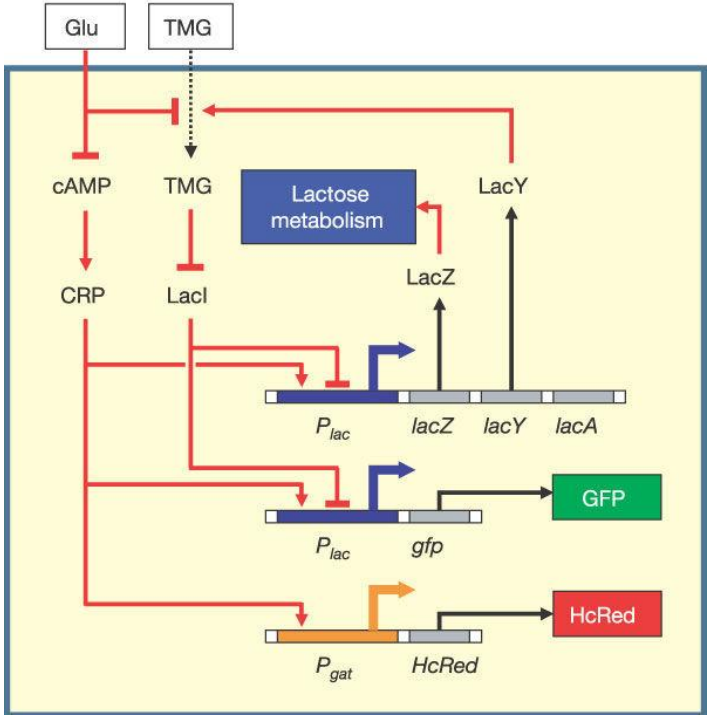


Figure: (Ozbudak et al.)

Red arrow - activation
Red blunt end - inhibition
Black arrow - protein creation
Dotted arrows - uptake across cell membrane

Modeling the *lac* system

- Equation S1 (Ozbudak et al.) - Active fraction of LacI:

$$\frac{R}{R_T} = \frac{1}{1 + (x / x_0)^n}$$

x: intracellular TMG concentration
R_T: total concentration of LacI tetramers
R: concentration of active LacI
x_0: half-saturation concentration
n: Hill coefficient, extensive experimental evidence shows it is approximately 2

- R/R_T is a decreasing sigmoidal function of x
  - Binding of TMG disrupts LacI activity; higher TMG occupancies cause further impairment

Modeling the *lac* system

- Equation S2 (Ozbudak et al.) - Rate of generation of LacY:

$$\tau_y \frac{dy}{dt} = \alpha \frac{1}{1 + R/R_0} - y$$

y: concentration of LacY (lactose permease) in green fluorescence units

τ_y: time constant

α: lac expression level that would be obtained if every repressor molecule were inactive

R: concentration of active LacI

R_0: half-saturation concentration

- Repression factor is defined as $\rho = 1 + R/R_0$
  - Repression factor describes how tightly LacI may regulate *lac* expression
- Decreasing hyperbolic function of R with maximal value α

Modeling the *lac* system

- Equation S3 (Ozbudak et al.)- Rate of entry of TMG concentration into cell:

$$\tau_x \frac{dx}{dt} = \beta y - x$$

x: intracellular TMG concentration
y: concentration of LacY (lactose permease) in green fluorescence units
τ_x: time constant
β: The transport rate; it gives the TMG uptake rate per LacY molecule

- TMG enters the cell at a rate proportional to y, and is similarly depleted in a first order reaction with time constant τ_x

Note: In the cell, TMG inactivates LacI and completes the feedback loop

Modeling the *lac* system

- Three equations (Ozbudak et al.):

$$\frac{R}{R_T} = \frac{1}{1 + (x / x_0)^n} \tag{S1}$$

$$\tau_y \frac{dy}{dt} = \alpha \frac{1}{1 + R / R_0} - y \tag{S2}$$

$$\tau_x \frac{dx}{dt} = \beta y - x \tag{S3}$$

Modeling the *lac* system

- Combine the three equations to obtain steady state result (Equation S4-(Ozbudak et al.)):

$$y = \alpha \frac{1 + (\beta y)^2}{\rho + (\beta y)^2}.$$

- ρ, α, and β are arbitrary functions of the external inputs, glucose (G) and TMG (T) levels
- As we vary these parameters, the system generates either one or two stable fixed points, with saddle node bifurcations separating these two behaviors

Modeling the *lac* system

- Rewrite Equation S4 as a cubic equation:

$$y^3 - \alpha y^2 + (\rho / \beta^2) y - (\alpha / \beta^2) = 0 . \qquad \text{(S5)}$$

- Theoretically, a generic cubic function with two identical roots has the form:

$$\begin{aligned} &(y-a)(y-a)(y-\theta a) \quad = \\ &y^3 - (2+\theta) a y^2 + (1+2\theta) a^2 y - \theta a^3 \end{aligned} \qquad \text{(S6)}$$

Note: θ = dimensionless ratio of roots
(Ozbudak et al.)

Modeling the *lac* system

- Compare coefficients and find:

$$\rho = (1+2\theta)(1+2/\theta),$$
$$\alpha\beta = (2+\theta)^{3/2}/\theta^{1/2}.$$

(Ozbudak et al.)

- These parametric equations describe the boundary of the bistable region (see figure to the right)
  - “Switching boundaries”

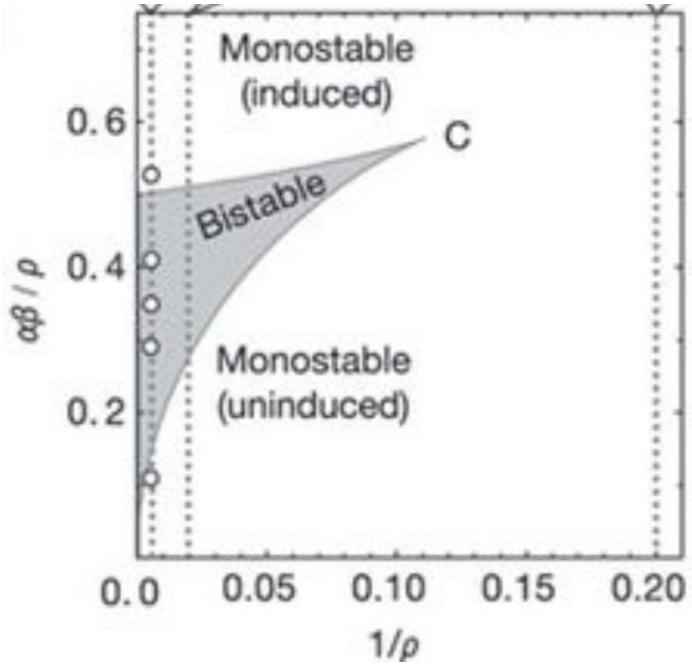


Figure: (Ozbudak et al.)

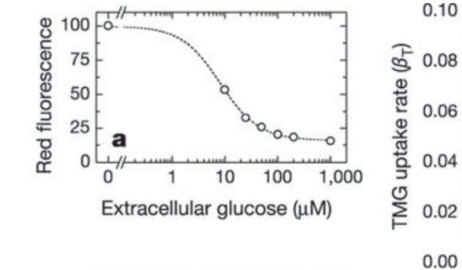
Measuring network parameters

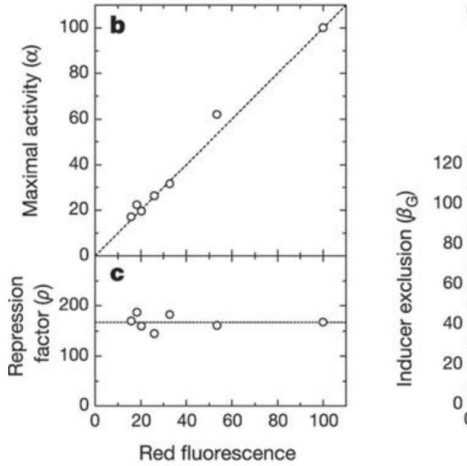
α - *lac* expression level obtained if every repressor molecule were inactive (maximum induction)

ρ (repression factor) - ratio of maximal to basal (read: every repressor molecule is active) activity

β (transport rate) - TMG uptake rate per LacY molecule

measured *in vivo*





Figures: (Ozbudak et al.)

Measuring network parameters

- Apply saddle node condition at each switching threshold (on boundary of bistable region)
  - Done separately at ON and OFF regions
  - Determine complete functional dependence on G and T (glucose and TMG levels, respectively)
- CAVEATS:
  - α 15% higher at OFF threshold
  - Large error in calculation of rho at OFF threshold due to low fluorescence values; estimate α and ρ at ON threshold alone
  - Decompose net TMG uptake rate as:

$$\beta(T,G) = \beta_T(T)\beta_G(G)$$

(Ozbudak et al.)

Measuring network parameters

- Caveats cont'd
  - Assume power law for β_T and use least-squares fitting routine to extract functions β_T and β_G
- We find:

$$\alpha = \frac{84.4}{1+(G/8.1)^{1.2}} + 16.1, \ \rho = 167.1,$$

$$\beta_T = (1.23\times10^{-3})T^{0.6}, \ \beta_G(G>10) \cong 65.$$

- [G]=[T]=ԟM

Equations: (Ozbudak et al.)

Phase diagrams: hysteresis vs. graded response

Wild-type network phase diagram:

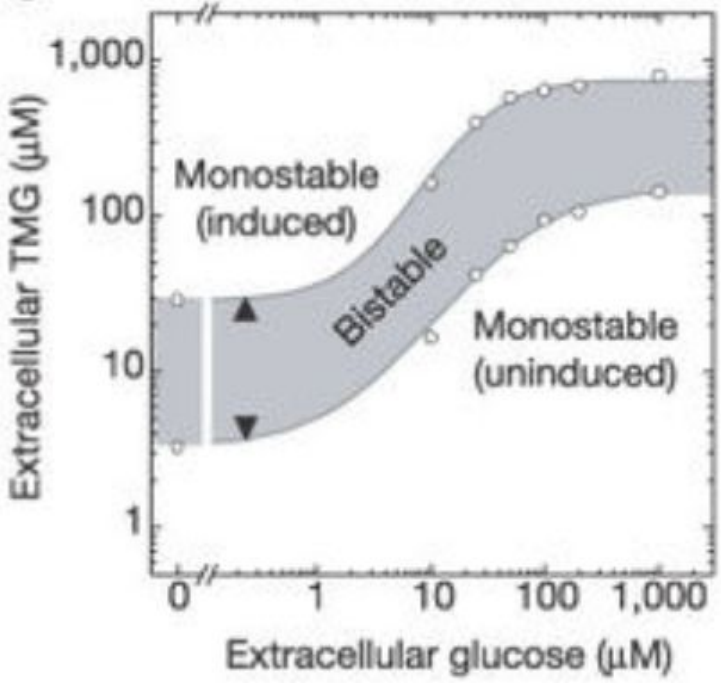


Figure: (Ozbudak et al.)

Theoretical phase diagram:

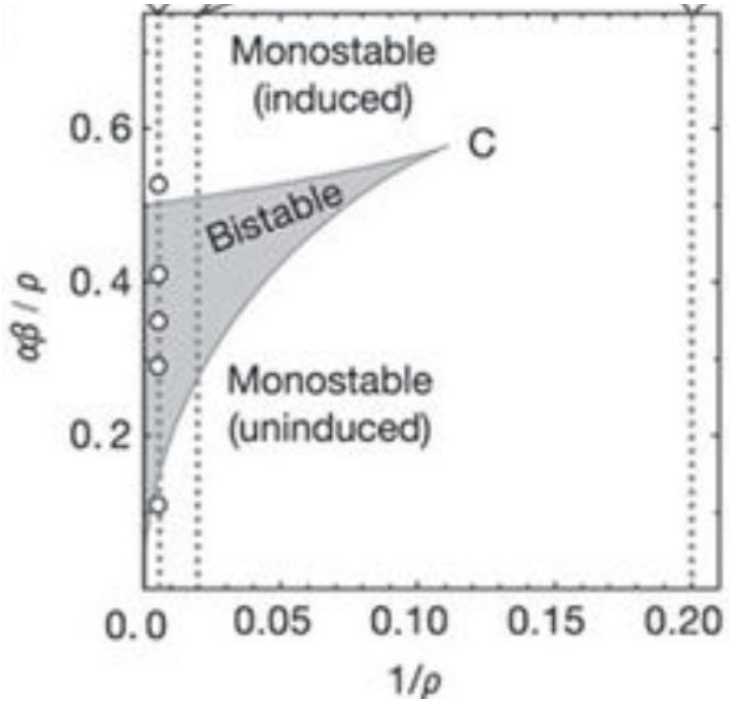


Figure: (Ozbudak et al.)

Phase diagrams: hysteresis vs. graded response

Wild-type network phase diagram:

- Maps out compete range of glucose and TMG levels over which system is bistable
- *lac* induction always takes place hysteretically
- Cells increase expression levels discontinuously as switching threshold is reached

Theoretical phase diagram:

- System response (moving from uninduced to induced) can occur in a graded fashion (white sections) or hysteretically (grey section)
- Expression levels of individual cells move continuously between low and high values
- Predicted to occur when degree of operon repression (rho) is decreased BELOW wild-type levels
    - Repression factor (and region of bistability) decreases to critical point at factor of 9
    - Graded behavior occurs beyond cusp

Current Progress

- Exploration of the parametric equations that describe the boundary of the bistable region
- Currently verifying plots based off of dynamic equations
- Developing general Matlab framework

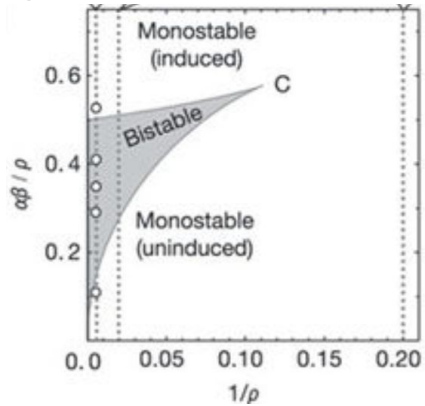
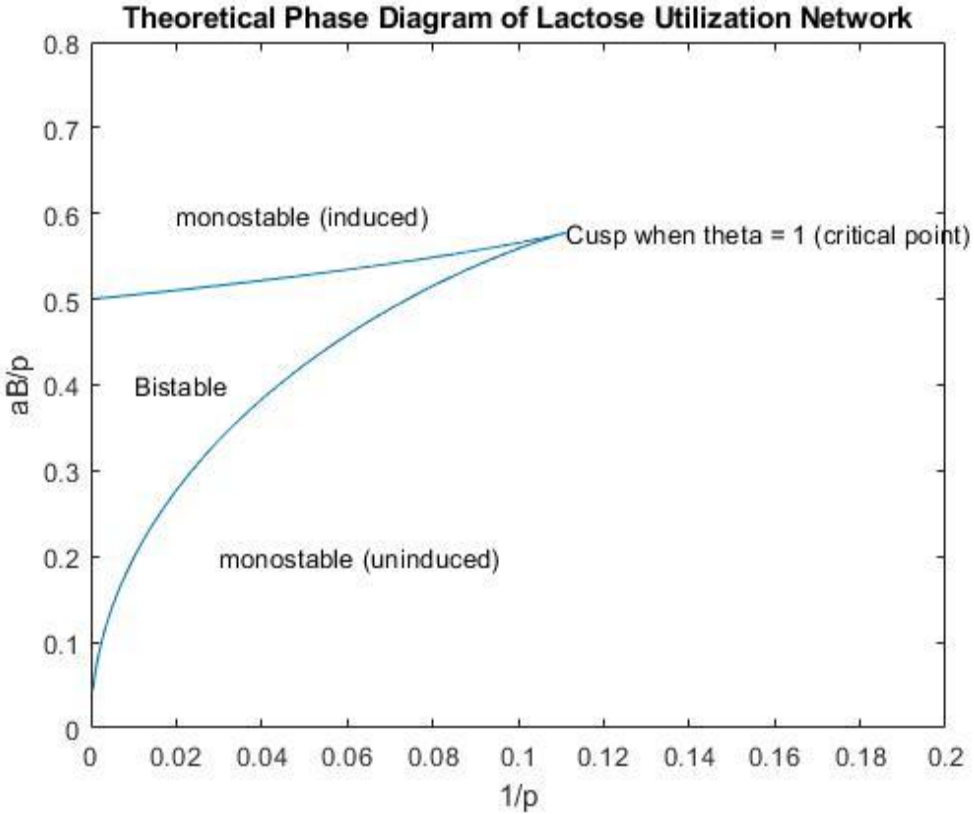


Figure: (Ozbudak et al.)



Current Progress

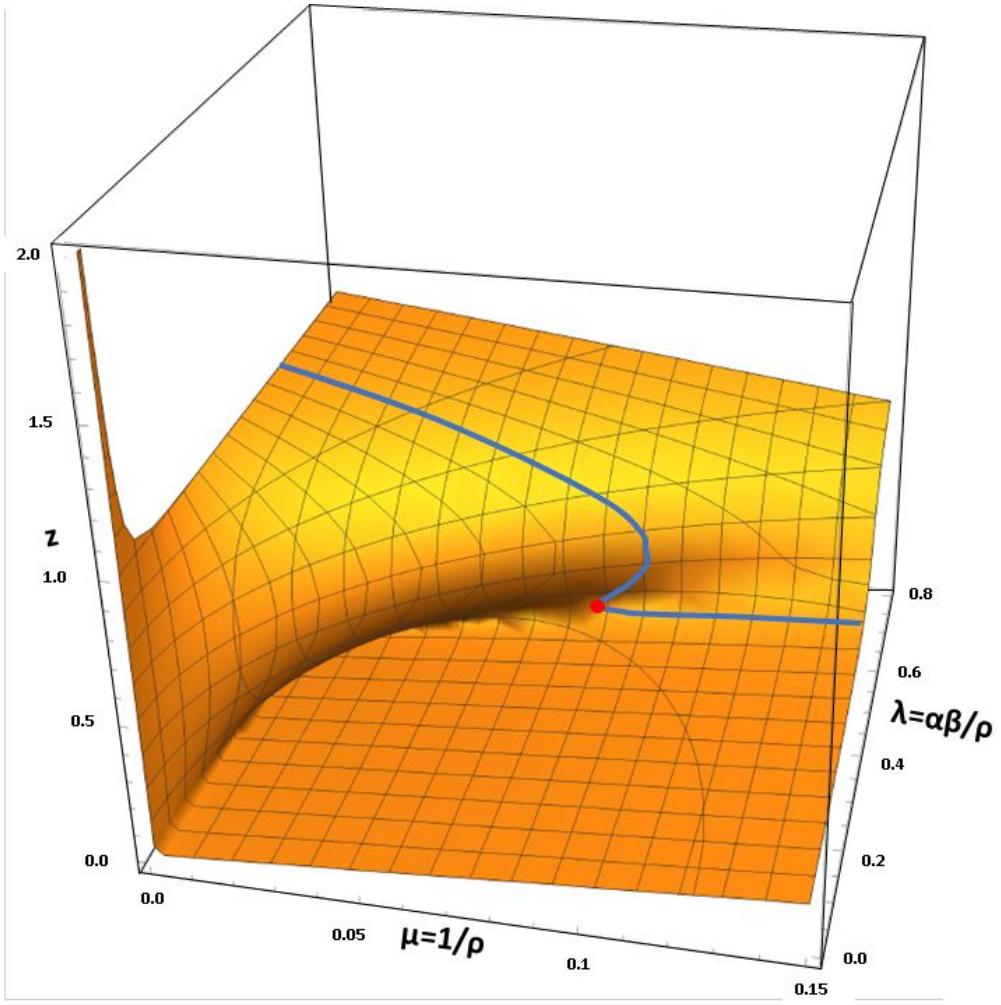
- Red Dot - Inflection point
- Blue Line: Trajectory as μ is changed

Variable Change: $y = \alpha z$

$$z = \frac{\frac{1}{\rho^2} + (\frac{\alpha\beta}{\rho} z)^2}{\frac{1}{\rho} + (\frac{\alpha\beta}{\rho} z)^2}$$

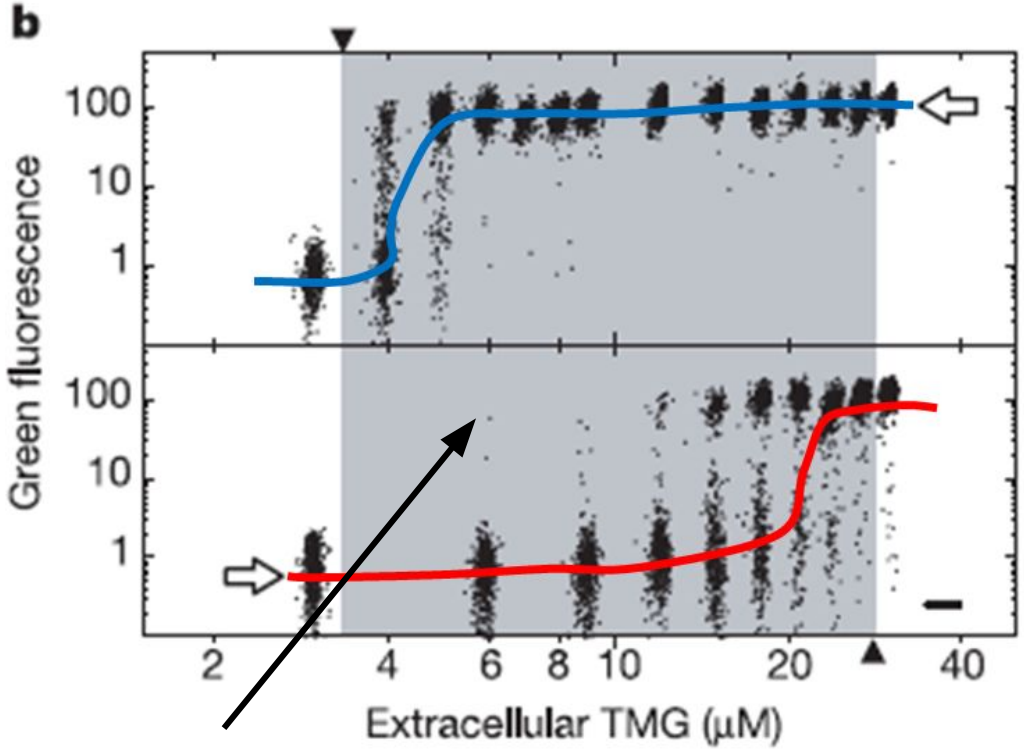
Redefine variables: $\mu = \frac{1}{\rho}$; $\lambda = \frac{\alpha\beta}{\rho}$

$$z = \frac{\mu^2 + (\lambda z^2)}{\mu + (\lambda z^2)}$$



Future work

- Generate data and model trajectories with differential equations
- Arrows indicate initial conditions
- Red : TMG > 30 μM to turn on initially **uninduced** cells
- Blue : TMG < 3 μM to turn off initially **induced** cells
- Proves hysteresis



Grey Region - Bistable

Figure: (Ozbudak et al.)

Future Work

- Discretize the model and implement them in MATLAB
- Recreate the phase diagrams using numerical analysis

Potential Ideas… “The discontinuous transition from low to high induction is analogous to a first order phase transition such as **evaporation in a liquid gas system**, with chemical noise instead of thermal noise driving stochastic transitions between these states”

We can attempt to validate this claim and show the similarities between the two systems

Implementation Plan

Roles:

- Lauren - Mathematical analysis and biological interpretation
- Katie - Mathematical analysis and biological interpretation
- Michael - Numerical Modeling (MatLab)
- Rob - Team Coordinator, Numerical Modeling (MatLab)

Sources

Ozbudak, Ertugrul M., Thattai, Mukund, Lim, Han N., Shraiman, Boris I., & van Oudenaarden, Alexander. Multistability in the lactose utilization network of *Escherichia coli. Nature.* **427**, 737-740 (2004).

Hansen, L. H., Knudsen, S. & Sorenson, S. J. The effect of the *lacY* gene on the induction of IPTG inducible promoters, studied in *Escherichia coli* and *Pseudomonas fluorescens*. *Curr. Microbiol.* **36**, 341-347 (1998).

Yagil, G. & Yagil, E. On the relation between effector concentration and the rate of induced enzyme synthesis. *Biophys. J.* **11**, 11-27 (1971).

SOS Math - Bifurcations
Khan Academy - *lac* operon
Nature Education - *lac* operon