Metabolic regulation
Using Evolution to understand genome structure and transcription regulation
Experimental and Modeling strategies

Experiments: use 'controlled conditions’

Mini-models: can study parameter space and 'choose' parameters based on outcome (fitting experiments)

Detailed models: use (MANY) measured / estimated ('reasonable’) parameters

minimal evolutionary optimization models
('what is it good for?) (bet-hedging)

Here use multilevel (evolutionary) modeling
to generate parameters and debug the above
Prototype gene regulation: Lac operon

The lac Operon and its Control Elements

CAP protein binding site

RNA polymerase

lac genes strongly expressed

Low glucose
Lactose available

High glucose
Lactose unavailable

lac genes not expressed

Low glucose
Lactose unavailable

High glucose
Lactose available

very low (basal) level of gene expression

An overview of the lac operon

Operon
LacZ
LacY

cAMP-CRP
LacI
B: β-galactosidase
P: permease
Lext: external lactose
Gext: external glucose
L: internal lactose
A: allolactose
G6P: glucose-6-phosphate

regulatory network
Lac Operon: Prototype bi-stability in gene regulation: classical mini-model, experiments

\[
\begin{align*}
R &= 1/(1 + A^n) \\
\frac{dM}{dt} &= c_0 + c(1 - R) - dM \\
\frac{dA}{dt} &= M L - \delta A - v M A \\
L &= 1.0; c = 1.0; c_0 = .05; \delta = .2; v = .25; n = 5)
\end{align*}
\]

bi-stability experimentally “verified”

cf Novick and Weiner 1957, Griffith 1968, Ozbudak et al 2004
Metabolic regulation in E. coli
Using Evolution to understand transcription regulation
Lac operon
experimental measurement of promotor function
Setty...Alon 2003

Not a simple AND function

“the wild-type region is selected to perform an elaborate computation in setting the transcription rate.”
measurements fitted to model of promoter function

\[ PA(A, C) = V_1 \frac{1 + V_2 A + V_3 R}{1 + V_4 A + V_5 R}, \]

where \( A \) stands for the allolactose concentration and \( C \) for the cAMP concentration and \( A \) and \( R \) are the fraction of active CRP and repressed LacI, respectively.

\[ A = \frac{(C/k_C)^n}{1 + (C/k_C)^n} \]  \hspace{1cm} (2)

\[ R = \frac{1}{1 + (A/k_A)^m}, \]  \hspace{1cm} (3)

where \( n \) and \( m \) are the Hill-coefficients of cAMP binding to CRP and allolactose binding to LacI. \( k_C \) and \( k_A \) are the dissociation constants for these reactions. Furthermore we have defined

\[ V_1 = (a\alpha + \gamma)/(1 + a) \]
\[ V_2 = d(b\beta + \gamma)/(a\alpha + \gamma) \]
\[ V_3 = \gamma c/(a\alpha + \gamma) \]
\[ V_4 = d(b + 1)/(a + 1) \]
\[ V_5 = c/(a + 1) \]  \hspace{1cm} (4)
\( V_1, \ldots, V_5 \) depend on 7 affinity parameters

\[ a = \frac{\text{RNAP}}{k_{\text{RNAP}}}, \] RNA-polymerase in units of its dissociation constant for binding to a free site.

\[ b = \frac{\text{RNAP}}{k_{\text{RNAP}}}, \] RNA-polymerase in units of its dissociation constant for binding to a site with bound CRP.

\[ c = \frac{\text{LacI}}{k_{\text{LacI}}}, \] the total LacI concentration in units of its dissociation constant for binding to its site.

\[ d = \frac{\text{CRP}}{k_{\text{CRP}}}, \] the total CRP concentration in units of its dissociation constant for binding to its site.

\[ \alpha, \] the transcription rate when RNA Polymerase is bound to the DNA, but CRP and LacI are not.

\[ \beta, \] the transcription rate when both RNA Polymerase and CRP are bound, but LacI is not bound to the DNA.

\[ \gamma, \] the “leakiness”, the transcription rate when RNA Polymerase is not bound to the DNA.
Table 1. *lac* model parameters that best fit the measurement using the GFP reporter plasmid (wild type) and putative mutants that have purer AND-like and OR-like gates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild type</th>
<th>AND</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m$</td>
<td>$4 \pm 0.6$</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>$n$</td>
<td>$2 \pm 0.4$</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$K_{\text{IPTG}}, \mu M$</td>
<td>$1.2 \pm 0.2$</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>$K_{\text{CAMP}}, \text{mM}$</td>
<td>$1.8 \pm 0.5$</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>$V_1$</td>
<td>$3.5 \pm 0.7$</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>$V_2$</td>
<td>$70 \pm 10$</td>
<td>70</td>
<td>1,700</td>
</tr>
<tr>
<td>$V_3$</td>
<td>$170 \pm 30$</td>
<td>2,000</td>
<td>15</td>
</tr>
<tr>
<td>$V_4$</td>
<td>$17 \pm 3$</td>
<td>17</td>
<td>400</td>
</tr>
<tr>
<td>$V_5$</td>
<td>$540 \pm 100$</td>
<td>7,000</td>
<td>50</td>
</tr>
</tbody>
</table>
more complex model of the lac operon

Wong et al 1997, adapted by van Hoek & Hogeweg 2006

\[
PA(A, C) \equiv V_1 \frac{1 + \frac{V_2(C/k_C)^n}{1 + (C/k_C)^n} + \frac{V_3}{1 + (A/k_A)^m}}{1 + \frac{V_4(C/k_C)^n}{1 + (C/k_C)^n} + \frac{V_5}{1 + (A/k_A)^m}}
\]

\[
\frac{dM}{dt} = P(A, C) - (\gamma_M + \mu)M
\]

\[
\frac{dB}{dt} = k_B M - (\gamma_B + \mu)B
\]

\[
\frac{dP}{dt} = k_P M - (\gamma_P + \mu)P
\]
eqs determining operon activity

\[ PA(A, C) = \frac{V_1}{1 + V_2 \frac{(C/k_C)^n}{1+(C/k_C)^n}} + \frac{V_3}{1 + V_4 \frac{(C/k_C)^n}{1+(A/k_A)^m}} + \frac{V_5}{1 + V_6 \frac{(A/k_A)^m}{1+(A/k_A)^m}} \]

\[ \frac{dM}{dt} = PA(A, C) - (\gamma_M + \mu)M \]

\[ \frac{dB}{dt} = k_B M - (\gamma_B + \mu)B \]

\[ \frac{dP}{dt} = k_P M - (\gamma_P + \mu)P \]

\[ \frac{dL}{dt} = P \frac{k_{L,i}L_{ext}}{K_{L,i} + L_{ext}} - P \frac{k_{L,o}L}{K_{L,o} + L} - B \frac{(k_{c,L} + k_{L-A})L}{L + K_{m,L}} - (\gamma_L + \mu)L \]

\[ \frac{dA}{dt} = B \frac{k_{L-A}L}{L + K_{m,L}} - B \frac{k_{c,A}A}{A + K_{m,A}} - (\gamma_A + \mu)A \]
eqs determining further metabolism and cell growth\( (X) \)

\( \text{cell division if cell size} = 2*\text{basic size} \)

\[
\frac{dG}{dt} = \frac{k_{c,L}B \times L}{L + K_{m,L}} + \frac{k_{c,A}B \times A}{A + K_{m,A}} - \frac{k_{c,G}G}{G + K_{m,G}} - k_{G,o}(G - G_{ext}) - \mu G
\]

\[
\frac{dG6P}{dt} = \frac{k_{t,G}G_{ext}}{G_{ext} + K_{t,G}} + \frac{k_{c,G}G}{G + K_{m,G}} + \frac{k_{c,L}B \times L}{L + K_{m,L}} + \frac{k_{c,A}B \times A}{A + K_{m,A}} -
\]

\[
\frac{k_{G6P,R}G6P}{G6P + K_{G6P,R}} - \frac{k_{G6P,F}G6P^8}{K_{G6P,F}^8 + G6P^8} - \mu G6P
\]

\[
\frac{dC}{dt} = \frac{k_{s,C}K_{s,C}}{k_{G6P,G}G_{ext} + K_{s,C}} - (\gamma C + \mu)C
\]

\[
\frac{dATP}{dt} = \frac{Y_R \times k_{G6P,R} \times G6P}{G6P + K_{G6P,R}} + \frac{2k_{G6P,F} \times G6P^8}{K_{G6P,F}^8 + G6P^8} - BMC -
\]

\[
\frac{\mu_{max} \times GC \times ATP^4}{ATP^4 + K_{ATP}^4} - PC \times PA - \frac{k_{c,L}B \times L}{L + K_{m,L}} - \frac{k_{c,A}B \times A}{A + K_{m,A}}
\]

\[
\frac{dX}{dt} = \frac{\mu_{max} \times ATP^4}{ATP^4 + K_{ATP}^4}X
\]

Wong concluded: bistable switch
Table 1: All model parameters with their values.

<table>
<thead>
<tr>
<th>parameter</th>
<th>equation</th>
<th>value</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_C$</td>
<td>Eq. 2</td>
<td>evolvable, mM</td>
<td>initial value: $1.0 \times 10^{-5}$ mM</td>
</tr>
<tr>
<td>$n$</td>
<td>Eq. 2</td>
<td>evolvable</td>
<td>initial value: 4.0</td>
</tr>
<tr>
<td>$k_A$</td>
<td>Eq. 3</td>
<td>evolvable, mM</td>
<td>initial value: $5.5 \times 10^{-5}$ mM</td>
</tr>
<tr>
<td>$m$</td>
<td>Eq. 3</td>
<td>evolvable</td>
<td>initial value: 8.0</td>
</tr>
<tr>
<td>$a$</td>
<td>Eq. 4</td>
<td>evolvable</td>
<td>initial value: 1.0</td>
</tr>
<tr>
<td>$b$</td>
<td>Eq. 4</td>
<td>evolvable</td>
<td>initial value: 1.0</td>
</tr>
<tr>
<td>$c$</td>
<td>Eq. 4</td>
<td>evolvable</td>
<td>initial value: $1.0 \times 10^6$</td>
</tr>
<tr>
<td>$d$</td>
<td>Eq. 4</td>
<td>evolvable</td>
<td>initial value: 50</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Eq. 4</td>
<td>evolvable, mM/min</td>
<td>initial value: $1.1 \times 10^{-7}$ mM/min</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Eq. 4</td>
<td>evolvable, mM/min</td>
<td>initial value: $2.2 \times 10^{-5}$ mM/min</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Eq. 4</td>
<td>evolvable, mM/min</td>
<td>initial value: $1.1 \times 10^{-9}$ mM/min</td>
</tr>
<tr>
<td>$\gamma_M$</td>
<td>Eq. 5</td>
<td>0.603/min</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$k_B$</td>
<td>Eq. 6</td>
<td>9.4 mM enzyme/(mM mRNA min)</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$\gamma_B$</td>
<td>Eq. 6</td>
<td>0.01/min</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$k_P$</td>
<td>Eq. 7</td>
<td>18.8 mM enzyme/(mM mRNA min)</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$\gamma_P$</td>
<td>Eq. 7</td>
<td>0.01/min</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$k_{Lac,in}$</td>
<td>Eq. 8</td>
<td>2148 mmol lactose/(mmol permease min)</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$K_{Lac,in}$</td>
<td>Eq. 8</td>
<td>0.26 mM</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$k_{Lac,out}$</td>
<td>Eq. 8</td>
<td>2148 mmol lactose/(mmol permease min)</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$K_{Lac,pat}$</td>
<td>Eq. 8</td>
<td>0.26 mM</td>
<td>unlike Wong et al. (2), intracellular concentrations are in mM</td>
</tr>
<tr>
<td>$k_{Lac-Allo}$</td>
<td>Eq. 9</td>
<td>8460/min</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$K_{m,Lac}$</td>
<td>Eq. 9, Eq. 10</td>
<td>1.4 mM</td>
<td>Martinez-Billbao et al. (13), referred to by Wong et al. (2)</td>
</tr>
<tr>
<td>$k_{cat,Lac}$</td>
<td>Eq. 10</td>
<td>9540/min</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$\gamma_L$</td>
<td>Eq. 11</td>
<td>0.15/min</td>
<td>assumed, to get a significant bistable region, compare Yildirim and Mackey (4)</td>
</tr>
<tr>
<td>$k_{cat,Allo}$</td>
<td>Eq. 12</td>
<td>18000/min</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$K_{m,Allo}$</td>
<td>Eq. 12</td>
<td>0.28 mM</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$\gamma_A$</td>
<td>Eq. 13</td>
<td>0.15/min</td>
<td>assumed, to get a significant bistable region, compare Yildirim and Mackey (4)</td>
</tr>
<tr>
<td>$k_{cat,Glu}$</td>
<td>Eq. 14</td>
<td>11.5 mM/min</td>
<td>fitted with data of Hogema et al. (6)</td>
</tr>
<tr>
<td>$K_{m,Glu}$</td>
<td>Eq. 14</td>
<td>0.45 mM</td>
<td>fitted with data of Hogema et al. (6)</td>
</tr>
<tr>
<td>$k_{Glu,out}$</td>
<td>Eq. 15</td>
<td>0.003/min</td>
<td>fitted with data of Hogema et al. (6)</td>
</tr>
<tr>
<td>$k_{L,Glu}$</td>
<td>Eq. 15</td>
<td>45 mM/min</td>
<td>Wong et al. (2), Carlson and Sieren (11)</td>
</tr>
<tr>
<td>$K_{L,Glu}$</td>
<td>Eq. 15</td>
<td>0.015 mM</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>parameter</td>
<td>equation</td>
<td>value</td>
<td>comments</td>
</tr>
<tr>
<td>----------------------------</td>
<td>----------</td>
<td>----------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>$k_{GiF,Resp}$</td>
<td>Eq. 18</td>
<td>34 mM/min</td>
<td>assumed, saturated respiratory flux assumed for maximal glucose influx. Andersen and Von Meyenburg (10)</td>
</tr>
<tr>
<td>$K_{GiF,Resp}$</td>
<td>Eq. 18</td>
<td>0.5 mM</td>
<td>idem. Andersen and Von Meyenburg (10)</td>
</tr>
<tr>
<td>$k_{GiF,Frm}$</td>
<td>Eq. 19</td>
<td>200 mM/min</td>
<td>assumed, maximal fermentative flux is much larger than maximal respiratory flux. Andersen and Von Meyenburg (10)</td>
</tr>
<tr>
<td>$K_{GiF,Frm}$</td>
<td>Eq. 19</td>
<td>20 mM</td>
<td>assumed, fermentation saturates much slower than respiration. Andersen and Von Meyenburg (10)</td>
</tr>
<tr>
<td>$k_{syn,cAMP}$</td>
<td>Eq. 21</td>
<td>0.001 mM/min</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$K_{syn,cAMP}$</td>
<td>Eq. 21</td>
<td>1.0 mM/min</td>
<td>assumed, to have a large range of possible cAMP concentrations.</td>
</tr>
<tr>
<td>$\gamma_{cAMP}$</td>
<td>Eq. 21</td>
<td>2.1/min</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>$Y_{R_at}$</td>
<td>Eq. 22</td>
<td>32 mM ATP/mM</td>
<td>assumed equal to the ATP-yield of aerobic respiration.</td>
</tr>
<tr>
<td>BMC</td>
<td>Eq. 22</td>
<td>23.5 mM/min</td>
<td>Carlson and Sriend (11)</td>
</tr>
<tr>
<td>GC</td>
<td>Eq. 22</td>
<td>$7.28 \times 10^5$ mM</td>
<td>estimated with data of Carlson and Sriend (11)</td>
</tr>
<tr>
<td>PC</td>
<td>Eq. 22</td>
<td>$2.36 \times 10^5$ m</td>
<td>ATM/mM mRNA calculated assuming 3% growth cost at maximal activity, Koch (14). (for high cost a value ten times higher is used)</td>
</tr>
<tr>
<td>$\mu_{max}$</td>
<td>Eq. 23</td>
<td>0.0233/min</td>
<td>Wong et al. (2)</td>
</tr>
<tr>
<td>Q</td>
<td>Eq. 25, Eq. 26</td>
<td>0.00035</td>
<td>assumed</td>
</tr>
<tr>
<td>$\dot{D}$</td>
<td></td>
<td>0.0020($gridsize)^2$/min</td>
<td>assumed, scalable</td>
</tr>
<tr>
<td>$\Delta_\alpha$</td>
<td></td>
<td>0.075</td>
<td>assumed</td>
</tr>
<tr>
<td>$\Delta_\beta$</td>
<td></td>
<td>0.075</td>
<td>assumed</td>
</tr>
<tr>
<td>$\Delta_\gamma$</td>
<td></td>
<td>0.15</td>
<td>assumed</td>
</tr>
<tr>
<td>$\Delta_\delta$</td>
<td></td>
<td>0.15</td>
<td>assumed</td>
</tr>
<tr>
<td>$\Delta_\alpha$</td>
<td></td>
<td>0.075</td>
<td>assumed</td>
</tr>
<tr>
<td>$\Delta_\beta$</td>
<td></td>
<td>0.075</td>
<td>assumed</td>
</tr>
<tr>
<td>$\Delta_\gamma$</td>
<td></td>
<td>0.075</td>
<td>assumed</td>
</tr>
<tr>
<td>$\Delta_{k_A}$</td>
<td></td>
<td>0.15</td>
<td>assumed</td>
</tr>
<tr>
<td>$\Delta_{k_C}$</td>
<td></td>
<td>0.05</td>
<td>assumed</td>
</tr>
<tr>
<td>$\Delta_n$</td>
<td></td>
<td>0.5</td>
<td>assumed</td>
</tr>
<tr>
<td>$\Delta_m$</td>
<td></td>
<td>0.5</td>
<td>assumed</td>
</tr>
<tr>
<td>$V_{mRNA,max}$</td>
<td></td>
<td>$2.2 \times 10^{-5}$ mM/min</td>
<td>assumed, to have a realistic maximal lactose uptake rate.</td>
</tr>
</tbody>
</table>
Functionality of Lac-operon
Bistability?

- Most studied regulatory system
- Often considered as AND gate
  
  *ON if lactose and not glucose; otherwise OFF*
  
- Recent direct promoter measurements: more graded response
- Bistability? Exp ’seen’ and expected from minimodels and ’verified’ in more extensive parametrized models
- Many (all) parameters measured
  
  **However**
  
  May be orders of magnitude different
  
  *parameter curse (2)!*

Setty .... Alon 2003

Does such a promoter function evolve

**Does Bistability Evolve?**; alleviate parameter uncertainty

van Hoek and Hogeweg, BJ 2006 PLOS Comp biol 2007
“experimental setup” evolution of the Lac operon: timescales: metabolism, cell growth/division, prot. stab, environmental switches, evolution

- Adapt existing detailed quantitative model of lac operon dynamics (Wong et al 1997)
- use measured parameters EXCEPT for lac operon parameters
- evolve 11 lac-operon parameters
  DO NOT use dimension reduction!
  otherwise evolutionary lock-in

- Design environment! (“cover all possibilities”)
- global/aperiodic influx of lactose and glucose in medium, diffusion, scaling
- growth (dependent on ATP), division (2*size), decay (density dep; no ATP)
- encountered environments depend on dynamics! dynamics!

evolution as trick to cope with parameter uncertainty
initialize as a bistable switch
(because no bistable switch evolved...)

solid low glucose; dotted high glucose
Designing external environment coverage of environmental statespace, while response to environments.

**Glucose vs. lactose, at end of evolution**

**cAMP vs. allolactose, at end of evolution**
Evolution: how to observe parameter of individuals in pop. in time
Evolution: how to observe phenotypic features in time (4 extremes)
Evolution: how to observe
comparison of evolutionary outcomes

Ancestor trace!

Compete last common ancestors (n*)

Compete last populations (n*)

--> “BEST” evolved promoter function
'Best' evolved last common ancestor
deterministic intracellular dynamics; 11hr average influx regime
spatial pattern formation

OBSERVED (Setty 2003)  BEST EVOLVED LCA
Similar to measured promoter function
However NO bistability
What about experiments / prior modeling?

Conditions for bistability for artificial inducer VERY different from those for lactose.

\[ \lambda(C) \equiv \frac{PA(0, C)}{(m-1)^2} \left( \frac{(m+1)^2}{PA(\infty, C)} + 4m\zeta \right) < 1. \]

for lactose:

\[ \frac{PA(0, C)4m\zeta}{(m-1)^2} < 1. \]

for artificial inducer

\[ \frac{PA(0, C)}{(m-1)^2} \frac{(m+1)^2}{PA(\infty, C)} < 1. \]

Evolved promoter function bistable for artificial inducer!
evolved vs measured bistability for artificial inducer

measured (van Oudenaarden)  evolve (van Hoek)
Why avoid bistability
why ‘waste’ expression when no (low) Lactose available
Non-equilibrium: delays!

lines for different $\gamma$ values ($P(0,C)$)

(E.coli division time ca 1hr)
sensitivity to experimental design
cost of expression and frequency of environmental switching

high cost bistab at rare high glucose
fast switch : loss of regulation
Experimental support for evolutionary model


in supplementary material

During induction with lactose, as opposed to IPTG, TMG...... the steady state distribution after 4 hours of growth is always uni-modal, and we never observe hysteresis.
various responses for different sugars
suppressing catabolism enhances hysteresis

Afroz et al 2014
general model allowing the various behaviors
most important: inductie strength and catabolism

\[ R = R_t \frac{S}{k_0 + S} \]
active regulator

\[ \frac{dS}{dt} = \gamma + \alpha_1 \frac{S_0}{k_1 + S_0} T - \alpha_2 \frac{S}{k_2 + S} E - \frac{d_1 S}{\text{dilution of sugar}} \]
endogenous production
active transport of sugar
catabolism of sugar

\[ \frac{dT}{dt} = \beta_T + \alpha_3 \frac{R^n_1}{k_3 + R^n_1} - \frac{d_2 T}{\text{dilution of transporter}} \]
basal expression of transporter
induction of transporter by sugar

\[ \frac{dE}{dt} = \beta_E + \alpha_4 \frac{R^n_2}{k_4 + R^n_2} - \frac{d_3 E}{\text{dilution of enzyme}} \]
basal expression of enzyme
induction of enzyme by sugar
experimental support of fast evolutionary change, avoidance of bistability even relative to TMG

Bistability and Nonmonotonic Induction of the lac Operon in the Natural Lactose Uptake System

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ABSTRACT The \textit{Escherichia coli} lac operon is regulated by a positive feedback loop whose potential to generate an all-or-none response in single cells has been a paradigm for bistable gene expression. However, so far bistable \textit{lac} induction has only been observed using gratuitous inducers, raising the question about the biological relevance of bistable \textit{lac} induction in the natural setting with lactose as the inducer. In fact, the existing experimental evidence points to a graded rather than an all-or-none response in the natural lactose uptake system. In contrast, predictions based on computational models of the lactose uptake pathway remain controversial. Although some argue in favor of bistability, others argue against it. Here, we reinvestigate \textit{lac} operon expression in single cells using a combined experimental/modeling approach. To this end, we parameterize a well-supported mathematical model using transient measurements of LacZ activity upon induction with different amounts of lactose. The resulting model predicts a monostable induction curve for the wild-type system, but indicates that overexpression of the LacI repressor would drive the system into the bistable regime. Both predictions were confirmed experimentally supporting the view that the wild-type \textit{lac} induction circuit generates a graded response rather than bistability. More interestingly, we find that the \textit{lac} induction curve exhibits a pronounced maximum at intermediate lactose concentrations. Supported by our data, a model-based analysis suggests that the nonmonotonic response results from saturation of the LacI repressor at low inducer concentrations and dilution of Lac enzymes due to an increased growth rate beyond the saturation point. We speculate that the observed maximum in the \textit{lac} expression level helps to save cellular resources by limiting Lac enzyme expression at high inducer concentrations.
model and parameters used (literature + measured) ((slightly) different to previous model)

No bistability for measured parameters, but can be induced by over-expression of LacI. LacZ expression saturates, and by dilution LacZ concentration peaks at intermediate Le, because growth rate increases. Zander et al 2017
Indeed in the model evolution of lac operon avoids bistability by increasing repressed expression level (and even more so in stochastic version)

dotted: start (bistable); solid evolved stoch.; dashed evolved det.
conclusions
Evolutionary modeling to 'test' regular systems biology models/experiments

Evolutionary perspective helped to debug long held misconceptions which were prior “verified” theoretically AND experimentally

Evolutionary modeling powerful tool for alleviating parameter uncertainty

Evolutionary change in parameters very uninformative

Parameter uncertainty inherent in evolutionary context (parameter redundancy; condition dependent parameter change ("TRUE" parameters do not exist) )

Non-supervised modeling 'fits' better then fitted supervised models
Modeling gene regulation/signal transduction

Monster of Loch Ness syndrome

“quod erat demonstrandum”

evolution as trick to cope with parameter uncertainty
HOWEVER: “function” of bistability is often assumed increased population variability, and therewith rapid adaptation, GIVEN stochastic gene expression

Above results artifact of deterministic modeling?

study: bistability and stochasticity in the lac operon

cf Thattai & van Oudenaarden (2004): noise + bistability can be 'good' because it allows rapid switching due to population heterogeniety.

However: minimization of expression noise in essential genes (Fraser et al 2004)

But: Excessive stochasticity of promoter function measured in E. coli (Wolf & van Nimwegen 2016)
from deterministic to stochastic model of lac operon
only one (measured) parameter added

protein translation occurs in bursts:
geometrically distribution, average size 5 proteins
(Cai et al 2007)

model chance of burst proportional to \# mRNA

at cell division distribute proteins binomially over the cells
intrinsic vs extrinsic noise: experiments and model

extrinsic noise: cell cycle + intracellular inducer concentration (green)

intrinsic noise: difference in expression of 2 identical promoters in a single cell (red)

\[ N_{tot} = N_{ext} + N_{int} = \text{std/mean} \text{ in population (blue)} \]

IPTG as inducer

Lac as inducer

IPTG as inducer

noise relative to internal protein numbers relative to external IPTG

black: internal protein number
evolution of lac operon with stoch. prot. expression avoids bistability even more

dotted: start (bistable); solid evolved stoch.; dashed evolved det.
WHY?
long delay in induction in stoch model
when in bistable regime (i.e. low repressed expression)

red ext. lac; blue ext. gluc; green βGalactosidase (high/low)
Relative Growth rates of promotor functions in deterministic and stochastice models

all times resource pulses are different
low repressed expression 'better' when no lactose and vv thherefor compare growth-rates over time relative to deterministic, low repressed rates

green: deterministic, low repressed
black stoch. : high repressed rates
red stoch: low repressed rates
population heterogeneity in various model variants: deterministic vs stochastic; genetic vs one clone, spatial vs well mixed

deterministic
black: full model; red: well mixed; green 1 clone full model; blue 1 clone well mixed; note partial synchronization; yellow intrinsic noise

stochastic
<table>
<thead>
<tr>
<th>Model</th>
<th>Source of Heterogeneity</th>
<th>Regression</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stochastic</td>
<td>Black: spatial + genetic</td>
<td>−0.47</td>
<td>−0.85</td>
</tr>
<tr>
<td></td>
<td>Green: spatial</td>
<td>−0.37</td>
<td>−0.86</td>
</tr>
<tr>
<td></td>
<td>Red: genetic</td>
<td>−0.32</td>
<td>−0.58</td>
</tr>
<tr>
<td></td>
<td>Blue: no</td>
<td>−0.16</td>
<td>−0.16</td>
</tr>
<tr>
<td></td>
<td>Yellow: intrinsic noise</td>
<td>−0.51</td>
<td>−1.00</td>
</tr>
<tr>
<td>Deterministic</td>
<td>Black: spatial + genetic</td>
<td>−0.68</td>
<td>−0.81</td>
</tr>
<tr>
<td></td>
<td>Green: spatial</td>
<td>−0.67</td>
<td>−0.78</td>
</tr>
<tr>
<td></td>
<td>Red: genetic</td>
<td>−0.59</td>
<td>−0.71</td>
</tr>
<tr>
<td></td>
<td>Blue: no</td>
<td>−0.029</td>
<td>−0.025</td>
</tr>
</tbody>
</table>
Population heterogeneity can be smaller than intrinsic noise because of non-equilibrium circumstances (during decay of proteins no heterogeneous burstsizes)
conclusions

Bistability even more detrimental when stochasticity is taken into account

on induction: long waiting for large bursts.

role of stochasticity overestimated by considering genetically identical cells in a homogeneous environment in equilibrium non-equilibrium conditions can reduce population heterogeneity

large genetic heterogeneity in natural populations: fast adaptation to environmental condition

_interlocking of evolutionary and regulatory timescales!

Parameter uncertainty inherent in evolution
MOREOVER: LACI autoregulator!
Semsey et al 2013

![Diagram of chromosome and plasmid with regulatory elements](image)

<table>
<thead>
<tr>
<th>Binding state</th>
<th>Relative probability</th>
<th>LacI production</th>
<th>LacZYA production</th>
</tr>
</thead>
<tbody>
<tr>
<td>unbound</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$O1$ bound</td>
<td>$\varepsilon_1 I^*$</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>$O1-O2$ loop</td>
<td>$\varepsilon_2 I^*$</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>$O1-O3$ loop</td>
<td>$\varepsilon_3 I^*$</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
LacI autoregulation leads to smoother activation, and less variation in delays relative to constitutive expression.

**LacY expression**

**Delay in expression after switch**

![Graphs showing LacY expression and delay in expression after switch for deterministic and stochastic models.]

<table>
<thead>
<tr>
<th>External lactose concentration (nM)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^3$</td>
<td>0.0015</td>
</tr>
<tr>
<td>$10^4$</td>
<td>0.05</td>
</tr>
<tr>
<td>$10^5$</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**Table 2.** Mean ± standard deviations of turn-on and turn-off times obtained in the simulations shown in Figure 6

<table>
<thead>
<tr>
<th>LacI range: 30–90 molecules</th>
<th>LacI range: 10–30 molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (autoregulated)</td>
<td>WT (autoregulated)</td>
</tr>
<tr>
<td>Constant low</td>
<td>Constant low</td>
</tr>
<tr>
<td>Constant high</td>
<td>Constant high</td>
</tr>
<tr>
<td>Turn-on time (minutes)</td>
<td>Turn-on time (minutes)</td>
</tr>
<tr>
<td>354.2 ± 41.7</td>
<td>349.3 ± 44.2</td>
</tr>
<tr>
<td>330.5 ± 85.4</td>
<td>308.7 ± 103.7</td>
</tr>
<tr>
<td>368.8 ± 36.0</td>
<td>364.8 ± 38.3</td>
</tr>
<tr>
<td>Turn-off time (minutes)</td>
<td>Turn-off time (minutes)</td>
</tr>
<tr>
<td>363.7 ± 74.4</td>
<td>394.0 ± 128.6</td>
</tr>
<tr>
<td>468.9 ± 246.0</td>
<td>602.0 ± 392.5</td>
</tr>
<tr>
<td>348.4 ± 33.0</td>
<td>354.4 ± 50.5</td>
</tr>
</tbody>
</table>