Gene Regulation: Lecture 3b
July 29, 2009

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Qualitatively Distinct Phenotypes in the Design Space of Biochemical Systems

Outline

- **Challenges in relating genotype to phenotype**
  - **Hierarchy of systems**
  - **Phenotype of molecular systems?**
- Hand-crafted constructions of design space
  - Physiological gene circuits
  - Engineered gene circuits
- Generic constructions of design space
  - Proposal based on the power-law formalism
  - Simple pathway
  - Core gene circuit for regulation of λ lysogeny
- Summary

"The problems faced by pre- and post-genomic genetics are ... much the same -- they all involve bridging the chasm between genotype and phenotype."

-- Sydney Brenner, *Science* **287**: 2173 (2000).

Three Fundamental Unsolved Problems

- Relationship of the genotype to the molecular components of the organism
  - DNA sequence does not tell what *kind* of component is being encoded
  - DNA sequence does not tell the *quantitative* values of the parameters
- Relationship of the molecular components to the system that is the organism
  - Parts (and their relevant parameter values) don't tell us *how they should fit together*
  - Parts don't tell us *which of them constitute the system* in a given environmental context
- Relationship of the molecular system that is the organism to its phenotypic repertoire
  - System (and the quantitative interaction of all its parts) does not tell us *how many qualitatively distinct phenotypes*
  - System does not tell us the *relative fitness of the phenotypes*

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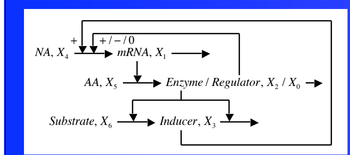
Design Space for Coupling of Expression in Elementary Gene Circuits

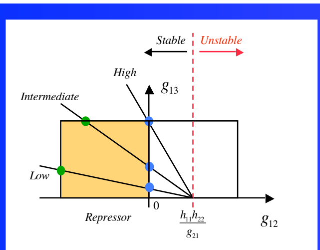


Boundary of kinetic orders

$g_{13}^{Max} = +2 \text{ or } +4 \quad g_{12}^{Max} = +2 \text{ or } +4 \quad g_{12}^{Min} = -2 \text{ or } -4$

Boundary of instability

$$g_{13} = \frac{h_{33}}{(g_{32} - h_{32})}\left\{\frac{h_{11}h_{22}}{g_{21}} - g_{12}\right\}$$



Line of equivalence

$$g_{13} = \frac{h_{33}L_{24}}{\left[g_{34} + (g_{32} - h_{32})L_{24}\right]}\left\{\frac{h_{11}h_{22}}{g_{21}} - g_{12}\right\}$$

Hlavacek & Savageau, *J. Mol. Biol.* **255**: 121 (1996)

Design Principle for the Coupling of Gene Expression in Elementary Circuits

Mode	Capacity	Predicted coupling
Positive	Small	Inverse & uncoupled
Positive	Large	Direct coupled
Negative	Small	Direct coupled
Negative	Large	Inverse & uncoupled

Hlavacek & Savageau, *J. Mol. Biol.* **248**: 739 (1995)
Hlavacek & Savageau, *J. Mol. Biol.* **266**: 538 (1997)

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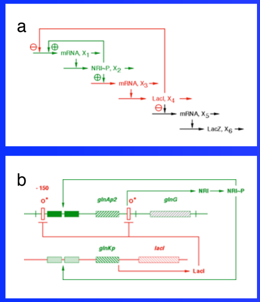
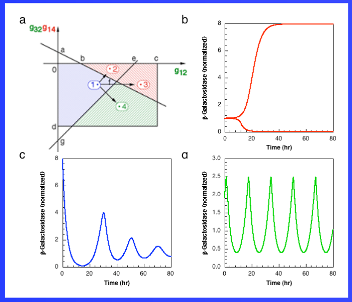
A Novel Circuit in *E. coli*

Kinetic Model and Genetic Construct

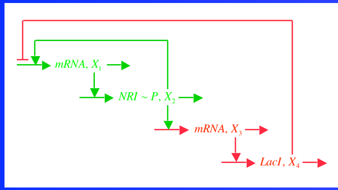


Design Space



Atkinson, *et al.*, *Cell* **113**: 597 (2003).

Synthetic Design Strategies



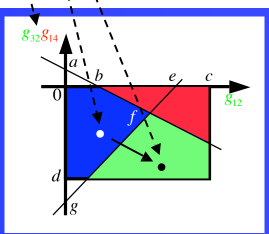
$a=(0,1)$ $\quad b=(1,0)$ $\quad c=(4,0)$ $\quad d=(0,-16)$

$$e=\left(\frac{(\beta_1+\beta_4)(\beta_2+\beta_4)}{\beta_1\beta_2},0\right)$$

$$f=\left(\frac{\beta_1\beta_2\beta_3+\beta_1\beta_2\beta_4+\beta_1\beta_3\beta_4+\beta_2\beta_3\beta_4}{\beta_1\beta_2\beta_3+\beta_1\beta_2\beta_4},-\frac{\beta_3\beta_4(\beta_1+\beta_2)}{\beta_1\beta_2(\beta_3+\beta_4)}\right)$$

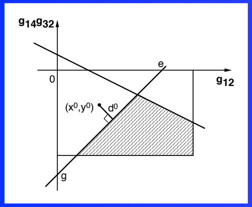
$$g=\left(0,-\frac{(\beta_1+\beta_2)(\beta_1+\beta_3)(\beta_1+\beta_4)(\beta_2+\beta_3)(\beta_2+\beta_4)(\beta_3+\beta_4)}{\beta_1\beta_2\beta_3\beta_4(\beta_1+\beta_2+\beta_3+\beta_4)^2}\right)$$

- Match model output to Experimental results
  - Location in design space
    - Strategy for re-engineering



Location and Movement in Design Space

Distance

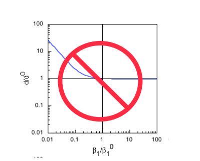


$$d=\frac{\sqrt{(y/x)^2\left[g_{14}g_{32}+(y/x)(x-1)\right]^2+\left[(y/x)g_{12}-g_{14}g_{32}-y\right]^2}}{1+(y/x)^2}$$

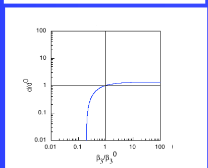
$$x=\frac{(\beta_1+\beta_4)(\beta_2+\beta_4)}{\beta_1\beta_2}$$

$$y=\frac{(\beta_1+\beta_2)(\beta_1+\beta_3)(\beta_1+\beta_4)(\beta_2+\beta_3)(\beta_2+\beta_4)(\beta_3+\beta_4)}{\beta_1\beta_2\beta_3\beta_4(\beta_1+\beta_2+\beta_3+\beta_4)^2}$$

Parameter Sensitivity



Life-time of activator mRNA



Life-time of repressor mRNA

Characteristics of Design Space

- Dimensional *compression* of parameter space
- All parameters included within aggregate factors
- Geometrical relationships
  - Constraints
  - Physical limits
  - Qualitative dynamics
  - *Qualitatively distinct* functional regimes
- Regions in design space correspond to qualitative distinct phenotypes

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Power-Law Formalism

$$\frac{dX_i}{dt} = \sum_{k=1}^{r} \alpha_{ik} \prod_{j=1}^{n} X_j^{g_{ijk}} - \sum_{k=1}^{r} \beta_{ik} \prod_{j=1}^{n} X_j^{h_{ijk}}$$

Canonical from Four Different Perspectives

- **Fundamental**
- **Local**
- **Piece-wise**
- **Recast**

Savageau, *Chaos* **11**: 142 (2001)

Generic Construction of Design Space

- Model of the system
  - Mass Action representation
  - Rational function representation
  - Other
- Recast into generalized mass action representation
  - Dominant terms produce a piecewise power-law representation
  - Bound on the number of phenotypic regions
- Local performance in each region described by an s-system
  - Signal amplification factors
  - Robustness
  - Response times
- Global performance described by boundaries
  - Regions with qualitative distinct phenotypes
  - Tolerance
  - Design principles

Savageau, *et al., PNAS* **106**: 6435 (2009).

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Two-Step Pathway

Dimensionless equation

$$\frac{dx}{d\tau} = \frac{x_S}{1 + x_S + x/\kappa} - \frac{\rho x}{1 + x + x_P}$$

$$X_s \underset{}{\overset{v_1}{\rightleftarrows}} X \underset{}{\overset{v_2}{\rightleftarrows}} X_p$$

$$x_S = X_S / K_S \qquad \kappa = K_I / K$$
$$x = X / K \qquad \rho = V_{Max,2} / V_{Max,1}$$
$$x_P = X_P / K_P \qquad \tau = \left(V_{Max,1} / K\right) t$$

Recast equation

$$x_S x_2^{-1} = \rho x_1 x_3^{-1}$$
$$x_2 = (1 + x_S) + x_1 / \kappa$$
$$x_3 = (1 + x_P) + x_1$$

Steady-state equation

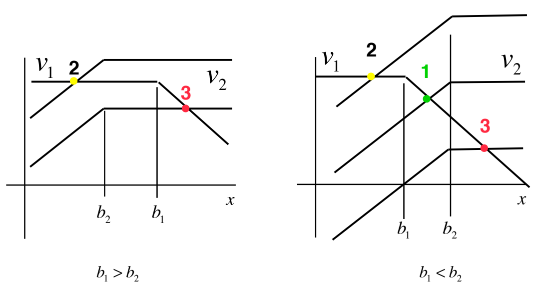
$$\frac{x_S}{1 + x_S + x/\kappa} = \frac{\rho x}{1 + x + x_P}$$

Bounds on # of regimes

$$T \le \prod_{i=1}^{n} P_i * N_i = 1 * 2 * 2 = 4$$

Steady-State Solutions for x: Three Qualitatively Distinct Cases

$$b_1 = \kappa(1 + x_S) \qquad b_2 = (1 + x_P)$$



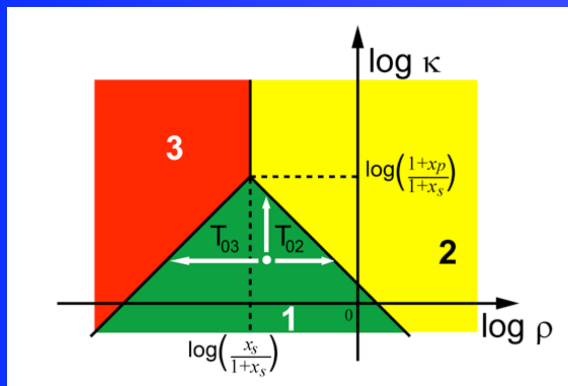
Piecewise Representation

$$\frac{dx}{d\tau} = \frac{x_S}{1 + x_S + x/\kappa} - \frac{\rho x}{1 + x + x_P}$$

$$X_s \overset{v_1}{\rightleftarrows} X \overset{v_2}{\rightleftarrows} X_p$$

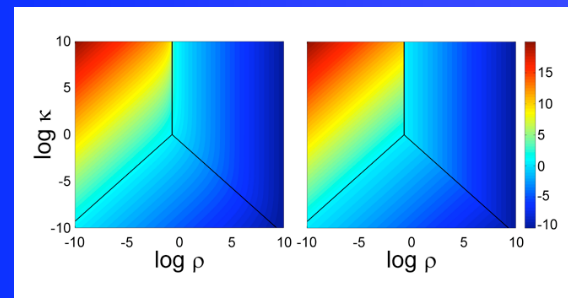
Solution 1	$\left[\frac{\kappa(1 + x_P) x_S}{\rho}\right]^{1/2}$	$x > \kappa(1 + x_S)$	$x < (1 + x_P)$
Solution 2	$\frac{x_S(1 + x_P)}{\rho(1 + x_S)}$	$x < \kappa(1 + x_S)$	$x < (1 + x_P)$
Solution 3	$\frac{\kappa x_S}{\rho}$	$x > \kappa(1 + x_S)$	$x > (1 + x_P)$
Solution 4	*undefined*	$x < \kappa(1 + x_S)$	$x > (1 + x_P)$

Design Space for a Pathway



Intermediate Concentration

Michaelis-Menten Piecewise Power Law



Design Principle

$$X_s \underset{}{\overset{v_1}{\rightleftarrows}} X \overset{v_2}{\rightleftarrows} X_p$$

Monofunctional

$$\frac{(1+x_P)x_S}{\kappa(1+x_S)^2} > \rho > \frac{\kappa x_S}{(1+x_P)}$$

Bifunctional

$$\frac{(1+x_P)}{\kappa(1+x_S)} > 1 \qquad \rho > \frac{(1+x_P)x_S}{\kappa(1+x_S)^2}$$

or

$$\frac{(1+x_P)}{\kappa(1+x_S)} < 1 \qquad \rho > \frac{x_S}{(1+x_S)}$$

κ small and ρ large

Implications

- 9-D *parameter* space compressed to 2-D *design* space
- Analysis within regimes is greatly simplified
- Phenotype of regime 1 most appropriate for a “mono-functional” intermediate
  - Minimal accumulation of intermediate avoids toxicity
  - Fast response time
  - Locally robust to changes in parameters
- Phenotype of regime 2 most appropriate for a “bi-functional” intermediate
  - Accumulation of intermediate facilitates functioning as a metabolic signal
  - Greater gain in flux when responding to input signals
- Phenotype of regime 3 has no appropriate function
- *Global tolerance* precisely defined as the parameter change necessary to cross the nearest boundary

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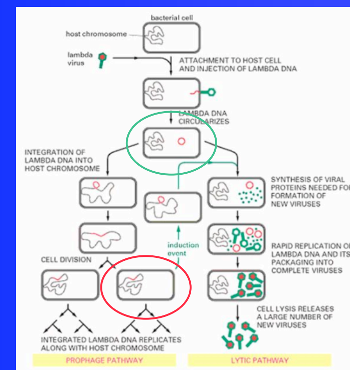
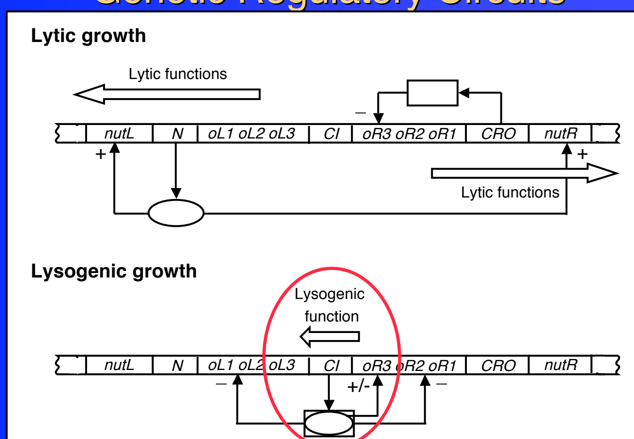
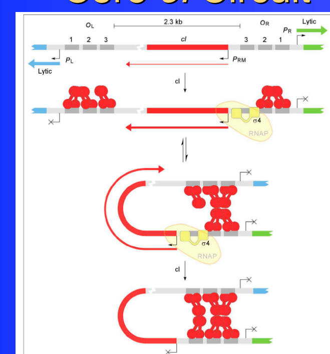
Phage Lambda Life Cycle



Genetic Regulatory Circuits



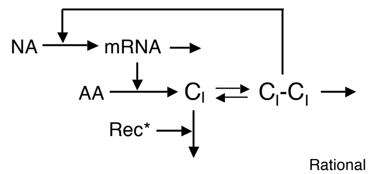
Molecular Interactions in the Core *cI* Circuit



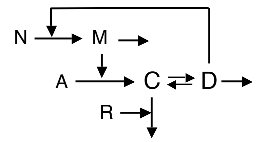
Dodd, *et al.*, *Current Opinion in Genetics & Development* **15**: 145 (2005).

Model Formulation

Model of the Lambda *cI* Gene Circuit



Rational Function Representation

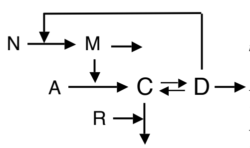


$$\frac{dM}{dt}=\left[\frac{\gamma_M K_D^p + D^p(\gamma_{MMax}+\gamma_M K_I^{-n} D^n}{K_D^p + D^p(1+K_I^{-n}D^n)}\right]-\delta_M M$$

$$\frac{dC}{dt}=\gamma_C M + 2\beta_D D - 2\gamma_D C^2 - \left[\frac{\delta_{CMax}R^a + \delta_C K_R^a}{R^a + K_R^a}\right]C$$

$$\frac{dD}{dt}=\gamma_D C^2 - \beta_D D - \delta_D D$$

Normalized Equations



Normalized Representation

$$\dot{m}=\left[\frac{\kappa^p + d^p(\sigma+\chi^{-n}d^n)}{\kappa^p + d^p(1+\chi^{-n}d^n)}\right]-m$$

$$\frac{1}{\rho_C}\dot{c}=m+2\frac{\beta_D}{\delta_{CMax}}d-\left[2\frac{\beta_D}{\delta_{CMax}}+\frac{\theta}{\alpha}\right]c^2-\left[\frac{(1+\alpha r^a)}{\sqrt{\alpha\theta}\phi(1+r^a)}\right]c$$

$$\frac{1}{\rho_D}\dot{d}=c^2-d$$

Normalized variables about steady state with maximal induction

$$\phi=\left(\frac{\gamma_M\gamma_C\gamma_D}{2\delta_M\delta_D(\beta_D+\delta_D)}\right)^{1/2} \qquad r=\frac{1}{K_R}R \qquad \sigma=\frac{\gamma_{MMax}}{\gamma_M} \qquad \rho_C=\sqrt{\alpha\theta}\phi\frac{\delta_C}{\delta_M}$$

$$m=\frac{\delta_M}{\gamma_M}M$$

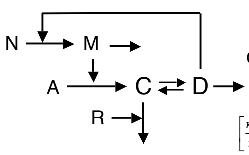
$$\kappa=\frac{\delta_M\delta_C}{\gamma_M\gamma_C}\alpha K_D \qquad \alpha=\frac{\delta_{CMax}}{\delta_C} \qquad \rho_D=\frac{\beta_D+\delta_D}{\delta_M}$$

$$c=\frac{\delta_M\delta_C}{\gamma_M\gamma_C}\sqrt{\alpha\theta}\phi C$$

$$\chi=\frac{\delta_M\delta_C}{\gamma_M\gamma_C}\alpha K_I \qquad \theta=\frac{2\delta_D}{\delta_C} \qquad \tau=\delta_M t$$

$$d=\frac{\delta_M\delta_C}{\gamma_M\gamma_C}\alpha D$$

Recast Steady-State Equations



Combining equations in steady state

$$\left[\frac{\kappa^p + d^p(\sigma+\chi^{-n}d^n)}{\kappa^p + d^p(1+\chi^{-n}d^n)}\right]=\left[\frac{\theta}{\alpha}\right]d+\left[\frac{(1+\alpha r^a)}{\sqrt{\alpha\theta}\phi(1+r^a)}\right]\sqrt{d}$$

Bound on total number of regions

$$T=\prod_{i=1}^{3}P_i * N_i$$
$$=(3*3)*(1*3)*(1*2)$$
$$=9*3*2=54$$

Recasting into a canonical GMA form and letting $d=x_1$

$$\theta\alpha^{-1}x_1 + x_1^{1/2}x_3^{-1} + \alpha x_1^{1/2}r^a x_3^{-1} = \kappa^p x_2^{-1} + \sigma x_1{}^p x_2^{-1} + \chi^{-n}x_1{}^{p+n}x_2^{-1}$$
$$x_2=\kappa^p + x_1{}^p + \chi^{-n}x_1{}^{p+n}$$
$$x_3=\alpha^{1/2}\theta^{1/2}\phi+\alpha^{1/2}\theta^{1/2}\phi r^a$$

Construction of System Design Space

Example of a Valid Solution

Piecewise equations

$$(\theta/\alpha)x_1 = \sigma x_1^p x_2^{-1} \quad x_2 = \chi^{-n} x_1^{p+n} \quad x_3 = \alpha^{1/2}\theta^{1/2}\phi$$

Solution

$$x_1 = d = \left[(\alpha/\theta)\sigma\chi^n\right]^{\frac{1}{n+1}} \quad c = \left[(\alpha/\theta)\sigma\chi^n\right]^{\frac{1}{2(n+1)}} \quad m = \left[(\theta/\alpha)\sigma^{1/n}\chi\right]^{\frac{n}{n+1}}$$

Conditions

$$(\theta/\alpha)x_1 > (\alpha/\theta)^{1/2}\phi^{-1}x_1^{1/2} \qquad (\theta/\alpha)x > (\alpha/\theta)^{1/2}\phi^{-1}x_1^{1/2}r^a$$

$$\sigma x_1^p > \kappa^p \qquad \sigma x_1^p > \chi^{-n}x_1^{p+n}$$

$$\chi^{-n}x_1^{p+n} > \kappa^p \qquad \chi^{-n}x_1^{p+n} > x_1^p$$

$$r < 1$$

Boundaries

$$\log\kappa < \frac{n+p}{p(n+1)}\log\sigma - \frac{n+p}{p(n+1)}\log(\theta/\alpha) + \frac{n(p-1)}{p(n+1)}\log\chi$$

$$\log r < \frac{3n+2}{2a(n+1)}\log(\theta/\alpha) + \frac{1}{2a(n+1)}\log\sigma + \frac{1}{a}\log\phi + \frac{n}{2a(n+1)}\log\chi$$

Example of an Invalid Solution

Piecewise equations

$$x_1^{1/2}x_3^{-1} = \kappa^p x_2^{-1} \quad x_2 = \kappa^p \quad x_3 = \alpha^{1/2}\theta^{1/2}\phi r^a$$

Solution

$$x_1 = d = \alpha\theta\phi^2 r^{2a} \quad c = \alpha^{1/2}\theta^{1/2}\phi r^a \quad m = 1$$

Conditions

$$r < \alpha^{-1/a} < 1 \qquad < \alpha/(\theta^3\phi^2 r^{2a})$$

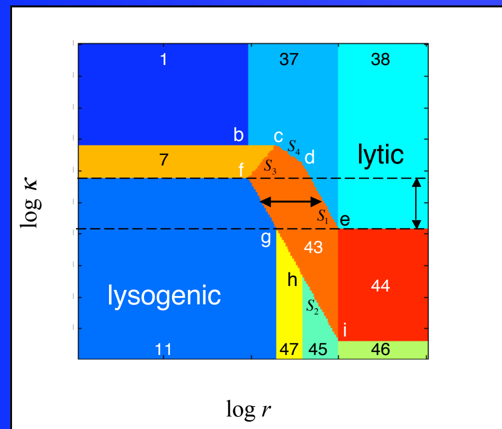
$$\kappa > \sigma^{1/p}x_1 \qquad \kappa > \chi^{-n/p}x_1^{(p+n0/p}$$

$$\kappa > x_1 \qquad \kappa > \chi^{-n/p}x_1^{(p+n)/p}$$

$$r > 1$$

Boundaries *Inconsistent*

Design Space



Landmarks in Design Space I

Intersections

$$b = \frac{1}{a}\log(\theta/\alpha) + \frac{1}{a}\log\phi, \frac{n+p}{p(n+1)}\log\sigma - \frac{n+p}{p(n+1)}\log(\theta/\alpha) + \frac{n(p-1)}{p(n+1)}\log\chi$$

$$c = \frac{3n+2}{2a(n+1)}\log(\theta/\alpha) + \frac{1}{2a(n+1)}\log\sigma + \frac{1}{a}\log\phi + \frac{n}{2a(n+1)}\log\chi,$$
$$\frac{n+p}{p(n+1)}\log\sigma - \frac{n+p}{p(n+1)}\log(\theta/\alpha) + \frac{n(p-1)}{p(n+1)}\log\chi$$

$$d = \frac{1}{2a}\log(\theta/\alpha) + \frac{1}{a}\log\sigma + \frac{1}{a}\log\phi - \frac{1}{2a}\log\chi, \log\chi$$

$$e = 0, 2\log\sigma + 2\log\phi + \log(\theta/\alpha)$$

Landmarks in Design Space II

Intersections

$$f = \frac{1}{a}\log(\theta/\alpha) + \frac{1}{a}\log\phi, \frac{1}{p}\log\sigma - \log(\theta/\alpha)$$

$$g = \frac{3n+2}{2a(n+1)}\log(\theta/\alpha) + \frac{1}{2a(n+1)}\log\sigma + \frac{1}{a}\log\phi + \frac{n}{2a(n+1)}\log\chi,$$
$$\frac{n+1-p}{p(n+1)}\log\sigma - \frac{2n+1}{(n+1)}\log(\theta/\alpha) - \frac{n}{(n+1)}\log\chi$$

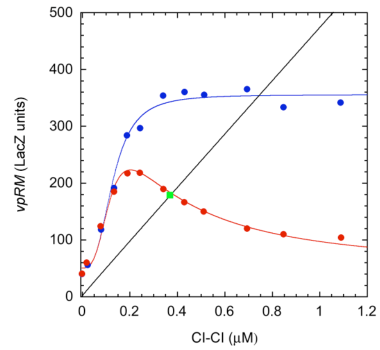
$$h = \frac{1}{2a}\log(\theta/\alpha) + \frac{1}{a}\log\sigma + \frac{1}{a}\log\phi - \frac{1}{2a}\log\chi, \log\chi - \frac{2p-1}{p}\log\sigma$$

$$i = 0, \frac{1}{p}\log\sigma + \log(\theta/\alpha) + 2\log$$

Slopes $s_1 = s_2 = -2a$ $\qquad s_3 = \frac{2a(p-1)}{p}$ $\qquad s_4 = -\frac{2a(p+n)}{p(2n+1)}$

Location in System Design Space

Rate of Transcription from P_{RM}

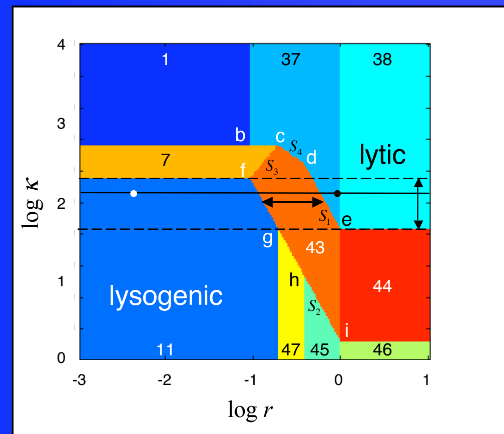


Dodd, *et al. Genes Dev.* **15**: 3013 (2001).

Parameters Values From Experimental Data

- Fitting model to data without inhibition
  - Capacity for regulation by CI — σ=7.1
  - Normalized K_M for activation — κ=130
  - Hill number for activation — p=3
- Fitting model to data with inhibition
  - Normalized K_I for repression — χ=230
  - Hill number for repression — n=1.5
- Fitting model to the normal operating point in lysogeny
  - Capacity for regulation by RecA* — α=215
  - Parameter characterizing dimerization of CI — ϕ=10

Design Space



Evaluation of Local Behavior

Local Criteria for Functional Effectiveness

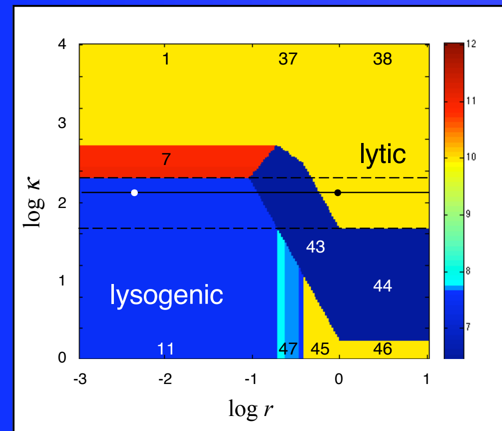
1. Maximize local robustness of D to parameter variation — $Min\left[\sum_i |S(D,p_i)|\right]$
2. Minimize response in D to variations in R — $Min\ L(D,R)$
3. Maximize local robustness of L(D,R) to parameter variation — $Min\left[\sum_i |S[L(D,R),p_i]|\right]$
4. Minimize response in M to variations in R — $Min\ L(M,R)$
5. Maximize local robustness of L(M,R) to parameter variation — $Min\left[\sum_i |S[L(M,R),p_i]|\right]$
6. Minimize response time — $Min\ |\mathrm{Re}[\lambda_{dominant}]^{-1}|$
7. Maximize local robustness of λ to parameter variation — $Min\left[\sum_i |S[\mathrm{Re}(\lambda)^{-1},p_i]|\right]$

Local Robustness in Each Phenotypic Region

Region	Criteria					
	L1	L2	L3	L4	L5	L6
Lysogenic regions (stable steady states)						
11	0.161±0.209*	0.222±0.282	0.000	0.000	0.000	0.000
47	0.209±0.248	0.293±0.267	0.500	0.117±0.301	0.750	0.088±0.282
45	0.862±0.878	0.133±0.340	2.000	0.067±0.249	0.000	0.000
46	0.667±0.863	0.133±0.340	0.000	0.000	0.000	0.000
Hysteretic regions (unstable steady states)						
7	0.277±0.410	0.474±0.657	0.000	0.000	0.000	0.000
43	0.323±0.325	0.555±0.497	0.400	0.147±0.376	1.200	0.077±0.276
44	0.230±0.308	0.381±0.433	0.000	0.000	0.000	0.000
Lytic regions (stable steady states)						
1	0.267±0.442	0.133±0.340	0.000	0.000	0.000	0.000
37	0.862±0.878	0.133±0.340	2.000	0.067±0.249	0.000	0.000
38	0.667±0.863	0.133±0.340	0.000	0.000	0.000	0.000

* Mean ± standard deviation

Local Response Time, $\tau_{1/2}$ (min)



Evaluation of Global Behavior

Global Criteria for Functional Effectiveness

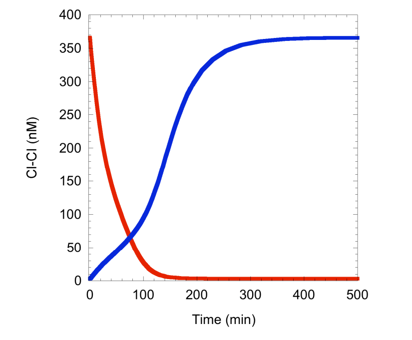
1. Maximize switching across the hysteretic region $Max\ \Delta_S = \alpha^2 / \left[\theta^2 \phi^2 \sigma^{(2p-1)/p}\right]$
2. Maximize robustness of this operation $Max\left[\sum_i \left|S(\Delta_S, p_i)\right|\right]^{-1}$
3. Maximize hysteretic buffer $Max\ \Delta_H = \sigma^{[(2p-1)/(2pa)]}$
4. Maximize robustness of buffer $Max\left[\sum_i \left|S(\Delta_H, p_i)\right|\right]^{-1}$
5. Maximize switching speed $Max\ \tau_S^{-1}$
6. Maximize Robustness of switching speed $Max\left[\sum_i \left|S(\tau_S, p_i)\right|\right]^{-1}$
7. Maximize global tolerances in best phenotypic region $Max\left[T_{low}, T_{high}\right]$

Global Tolerances for the Lysogenic Phenotype

mRNA		Protein	
Parameter	Tolerance	Parameter	Tolerance
γ_M	[∞,4.2]*	δ_C	[∞,4.2]
$\gamma_{M\,\max}$	[2.4,1.7]	$\delta_{C\,\max}$	[1.6,38]
K_D	[2.8,4.1]	K_R	[38,∞]
K_I	[11,2.4]	R	[∞,38]
p	[∞,∞]	a	[4.8,∞]
n	[∞,∞]	γ_C	[2.4,1.7]
δ_M	[1.7,2.4]	γ_D	[∞,2.8]
δ_D	[11,2.1]	β_D	[2.8,1500]

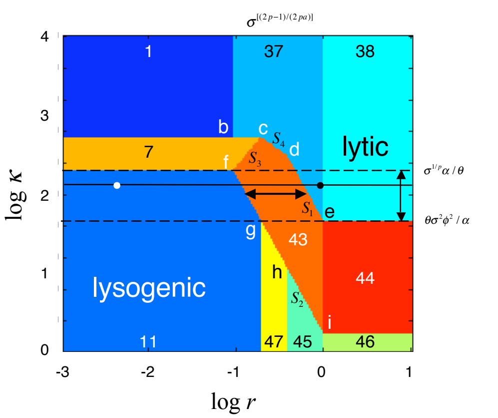
* [fold decrease,fold increase]

Switching Times to Turn ON (blue) and Turn OFF (red) the *cI* Gene Circuit



5 X $\tau_{1/2}$

Design Space



Additional Influences on the Core *cI* Circuit

- Effects promoting induction (Atsumi & Little, 2006)
  - CRO is unnecessary for induction (Svenningsen, et al., 2005).
  - But it does lower the maximal rate of *cI* transcription γ_{Mmax}, which reduces the threshold level of RecA* needed for induction
- Effects promoting lysogeny (Kourilsky & Knapp, 1974)
  - Multiplicity of infection leads to elevated CII levels (Kobiler, et al., 2005)
    - This increases the maximal rate of *cI* transcription γ_{Mmax}
    - Thus, raising the boundary between the lytic and lysogenic regions
  - Slower growth rates lead to an increase in CI
    - This is a result of lowering the rate constant for dilution δ_C
    - Again, expanding the lysogenic regions at the expense of the lytic regions

Implications

- 15-D *parameter* space compressed to 2-D *design* space
- Analysis within regimes is greatly simplified
- Phenotypes representing the stable steady state with induction
- Phenotypes representing multiple steady states with an hysteretic response
- Phenotypes representing less appropriate steady states for the lysogenic state
- Phenotypes representing most appropriate steady state for the lysogen
  - Locally robust to parameter changes
  - Fast response times
  - Large global tolerance to parameter changes
  - Fast switching times for induction and integration
- *Qualitative* prediction of hysteretic region
- Suggestions for design principles
  - Δ S
  - Δ H
  - Cleavage only of the monomer
  - Induction much faster then integration

Summary

- Motivated by results from successful hand-crafted design spaces
- Proposal for a generic method of constructing design space
  - Design space as a dimensional compression of parameter space
  - Phenotypes associated with regions of design space
  - Bound on the number of qualitatively distinct phenotypes
  - Simple characterization of local behavior within regions
  - Fitness comparisons among phenotypes
  - Precisely defined boundaries between regions
  - Novel definition of **global tolerance** to parameter change
  - Facilitates identification of **system design principles**
- Foundation in algebraic geometry
  - All boundaries are straight lines in log space
  - Intercepts are linear in the logarithms of the rate constants
  - Slopes and intercepts are rational functions of the kinetic orders

Acknowledgements

- **Eberhard Voit**
- **William Hlavacek**
- **Armindo Salvador**
- **Pedro Coelho**
- **Dean Tolla**
- **Rick Fasani**

- **NIH, NSF, ONR**