Phenotypes in the Design Space of Biochemical Systems

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Outline

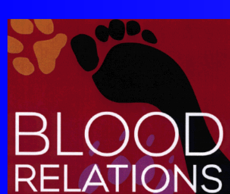
- Challenges in relating genotype to phenotype
  - Hierarchy of systems
  - Phenotype of molecular systems?
- Hand-crafted constructions of system design space
  - Physiological gene circuits
  - Engineered gene circuits
- Generic constructions of system design space
  - Proposal based on the power-law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

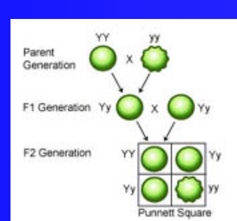
"Never, anywhere, have I seen so great a likeness in man or woman – but it is truly strange! This boy must be the son of Odysseus, Telékhos, the child he left at home that year the Akhaian host made war on Troy."

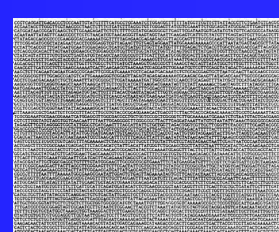
Homer (800 - 600 BC). *The Odyssey*, 4, 152-156

Hereditary Determinant



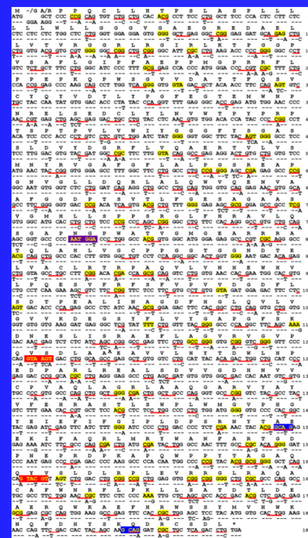







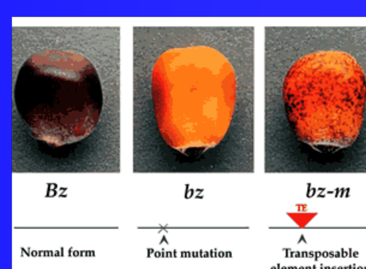
Genotype

DNA Structure

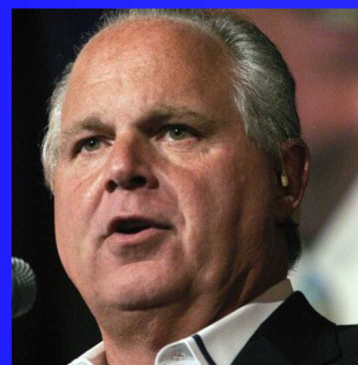


Genome Sequence

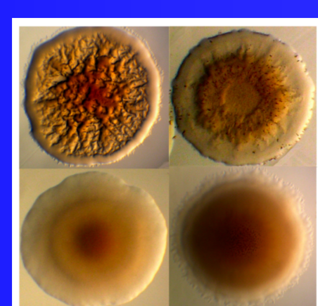
Plant Phenotypes



Animal Phenotypes



Microbial Phenotypes

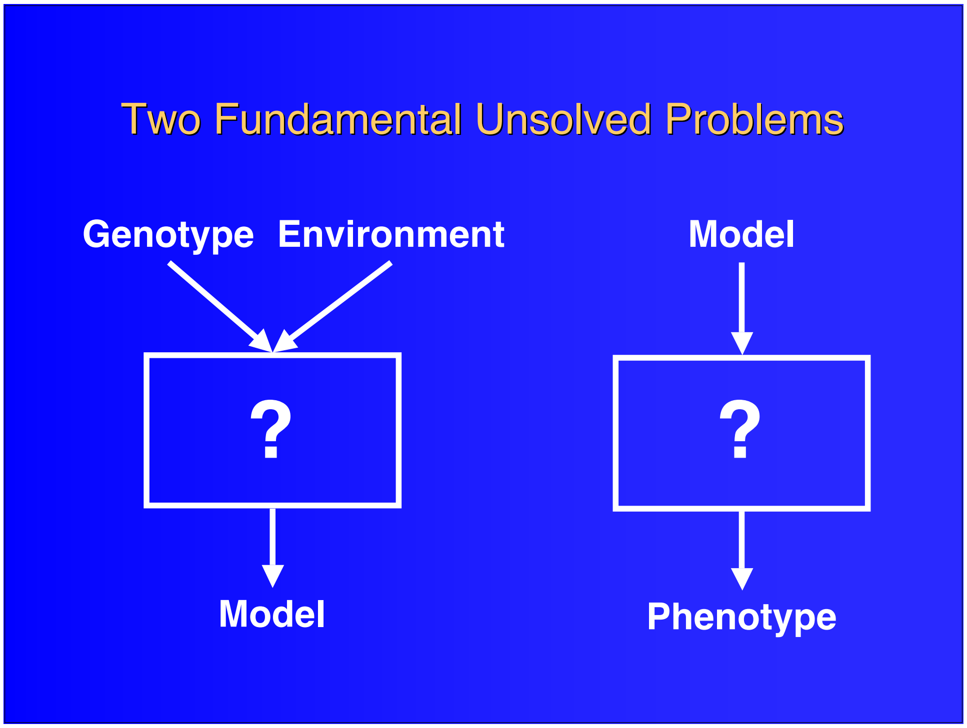


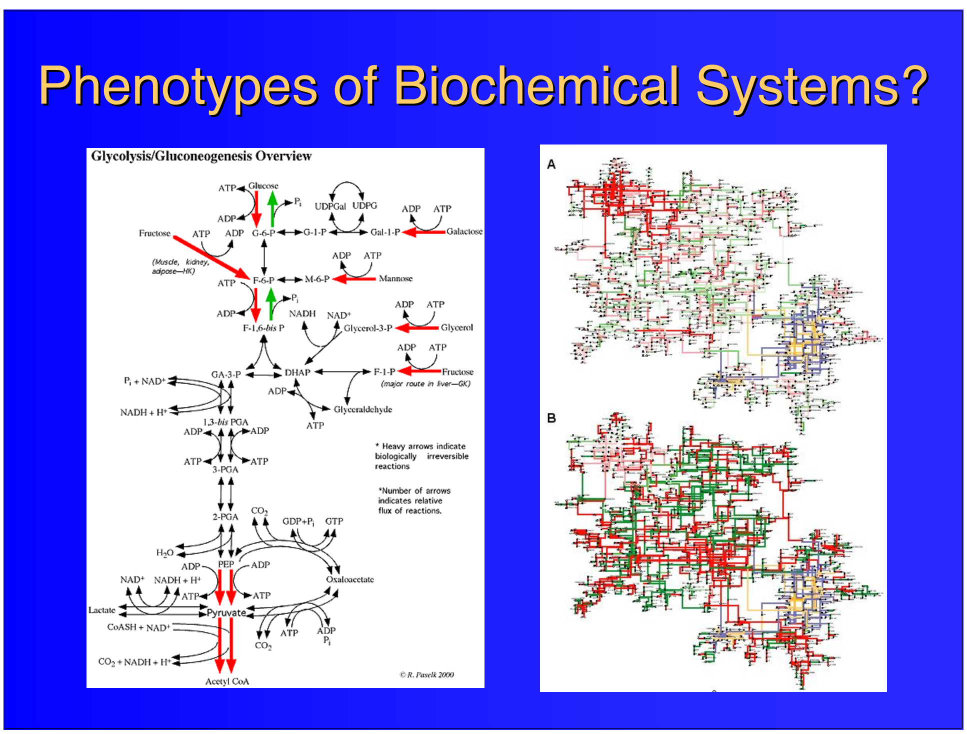
"The problems faced by pre- and post-genomic genetics are ... much the same -- they all involve bridging the chasm between genotype and phenotype."

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

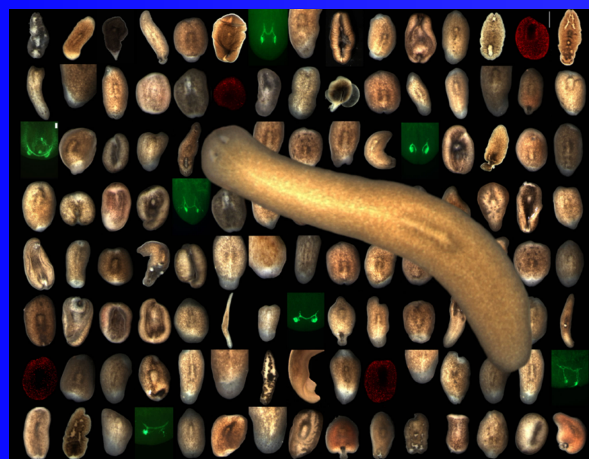
Function of Gene Circuitry

- Superficial answer
  - Genotype determined by the information encoded in the DNA sequence
  - Phenotype by the context-dependent expression of the genome
  - Circuitry interprets context and orchestrates expression
- Deeper answer
  - Hierarchy of systems
  - Phenotypes at each level
  - Diversity of design issues
  - Accident or rule


Two Fundamental Unsolved Problems
Genotype
Environment
?
Model
Model
?
Phenotype


Phenotypes of Biochemical Systems?
Glycolysis/Gluconeogenesis Overview
* Heavy arrows indicate biologically irreversible reactions
*Number of arrows indicates relative flux of reactions.
© R. Paselk 2000
A
B

How Many Phenotypes?



The normal freshwater planarian *Schmidtea mediterranea* is seen in the foreground gliding over a composite background of some of the 240 phenotypes (defects) generated by the RNA silencing screen.

Goals

- Develop a generic method for constructing the system design space
- Define qualitatively distinct phenotypes
  - Identification
  - Enumeration
- Analyze and compare their relative fitness
- Measure tolerance to global change from one phenotype to another

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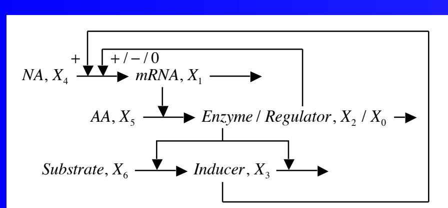
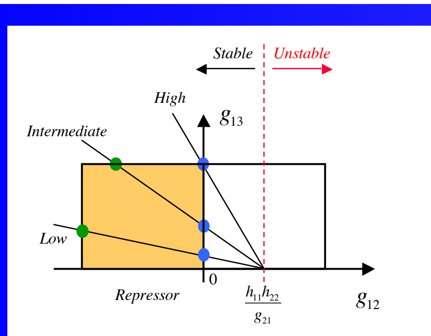
Design Space for Coupling of Expression in Elementary Gene Circuits





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

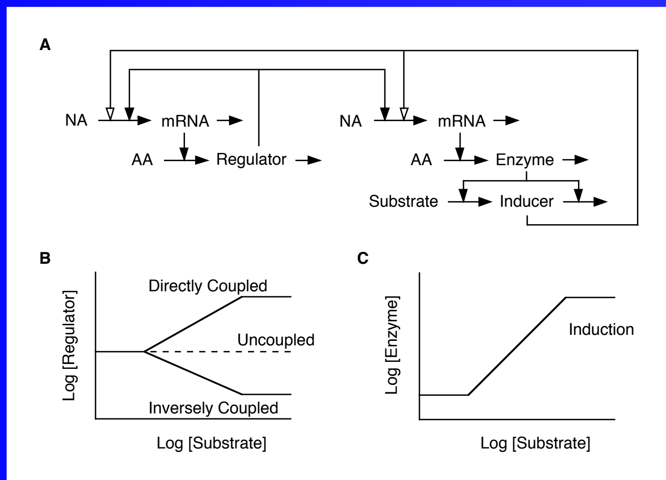
$$g_{13} = \frac{h_{33}}{(g_{32} - h_{32})} \left\{ \frac{h_{11} h_{22}}{g_{21}} - g_{12} \right\}$$

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.* **248**: 739 (1995)

Coupling of Gene Expression in Elementary Circuits



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.* **266**: 538 (1997)
Wall et al. *Nature Review Genetics* (2004)

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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Interlaced Levels of Description for a Chemical Reaction

Time/Number Scale: Small ↑ … ↓ Large

QM wave function	Discrete/Stochastic
Potential energy function	Continuous/Deterministic
Probability distribution function	Discrete/Stochastic
Rate law function	Continuous/Deterministic
Boolean function	Discrete/Deterministic

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)

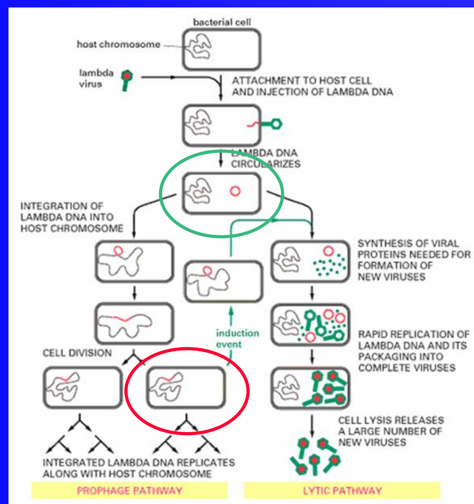
Generic Construction of Design Space

- 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

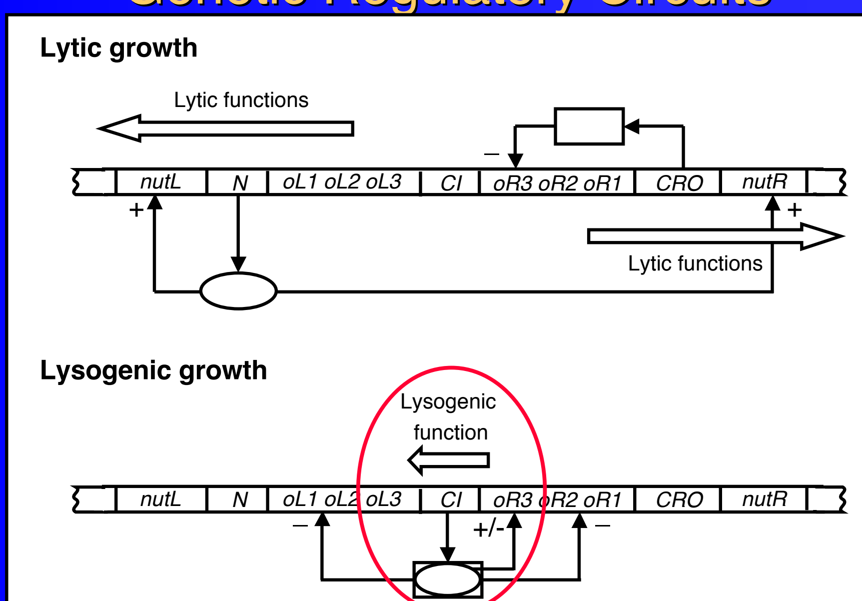
Savageau, *et al., PNAS* **106**: 6435 (2009).

Phage λ *cI* Gene Circuit

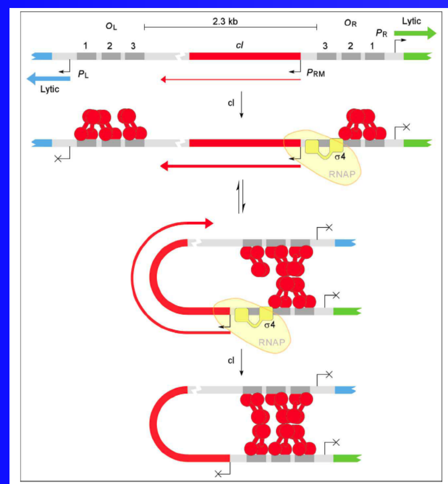
Phage Lambda Life Cycle



Genetic Regulatory Circuits

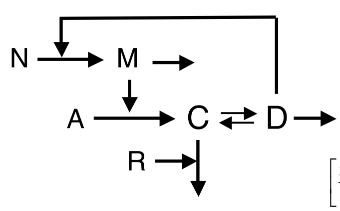


Molecular Interactions in the Core *cI* Circuit



Dodd, *et al.* (2005)   Vilar, J. M., and Saiz, L. (2005)

Model of The *cI* Gene Circuit and Its Recast 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

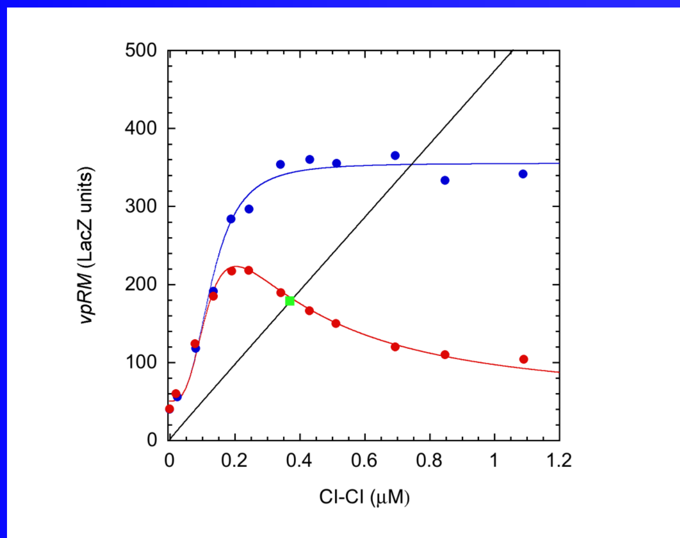
$$T = \prod_{i=1}^{3} P_i * N_i$$
$$= (3*3)*(1*3)*(1*2)$$
$$= 9*3*2 = 54$$

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

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

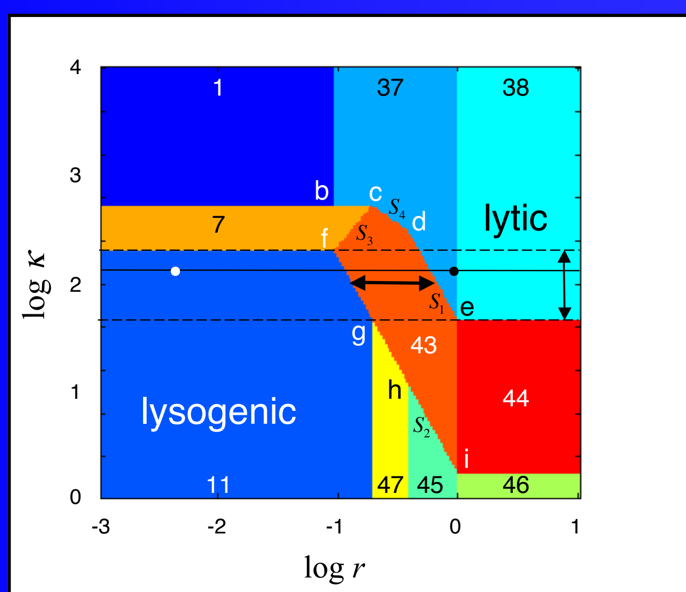
Savageau and Fasani, *FEBS Lett* **583**, 3914 (2009)

Rate of Transcription from P_{RM}



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

System Design Space



Evaluation of Local Behavior

Analytical Determination of Robustness

Robustness measured by parameter insensitivity

Parameter sensitivities defined as $S(V_i, p_j) = \frac{\partial V_i}{\partial p_j} \frac{p_j}{V_i}$

External equivalence implies $g_{13}{}^{u} = g_{13}{}^{p} \frac{h_{11} h_{22}}{h_{11} h_{22} - g_{12}{}^{p} g_{21}}$

Ratio for comparison $\frac{S(V_3, \beta_2)^{p}}{S(V_3, \beta_2)^{u}} = \frac{h_{11} h_{22}}{h_{11} h_{22} - g_{12}{}^{p} g_{21}} < 1 \text{ for } g_{12}{}^{p} < 0$

Conclusion: Perfectly coupled circuit with repressor control is more robust than the equivalent completely uncoupled circuit

Savageau, *Nature* **229**: 542 (1971)
Becskei & Serrano, *Nature* **405**: 590 (2000)

Locally Robust in Each Region

Analytical Results

Region	Criteria								
	$\Sigma \mid S(\bullet, p_i) \mid /N$			$L(\bullet, R)$			$\Sigma \mid S[L(\bullet, R), p_i] \mid /N$		
	D	*C*	*M*	*D*	*C*	*M*	*D*	*C*	*M*
Lysogenic regions (stable steady states)									
11	0.161±0.209*	0.141±0.164	0.187±0.247	0.000	0.000	0.000	[illegible]	0.000	0.000
47	0.292±0.255	0.180±0.144	0.318±0.309	-0.500	[illegible]	[illegible]	[illegible]	0.117±0.301	0.083±0.253
45	0.862±0.878	0.364±0.464	0.133±0.340	-2.000	[illegible]	[illegible]	0.067±0.249	0.067±0.249	0.000
46	0.667±0.863	0.267±0.442	0.133±0.340	[illegible]	[illegible]	0.000	0.000	0.000	0.000
Hysteretic regions (unstable steady states)									
7	0.277±0.411	0.205±0.227	[illegible]	[illegible]	0.000	0.000	0.000	0.000	0.000
43	0.291±0.300	[illegible]	[illegible]	0.400	0.200	1.200	0.147±0.376	0.147±0.376	0.080±0.251
44	0.238±0.310	[illegible]	[illegible]	0.000	0.000	0.000	0.000	0.000	0.000
Lytic regions (stable steady states)									
1	[illegible]	[illegible]	0.133±0.340	0.000	0.000	0.000	0.000	0.000	0.000
37	[illegible]	0.409±0.502	0.133±0.340	-2.000	-1.000	0.000	0.067±0.249	0.067±0.249	0.000
38	0.667±0.863	0.267±0.442	0.133±0.340	0.000	0.000	0.000	0.000	0.000	0.000

* Mean ± standard deviation

Evaluation of Global Behavior

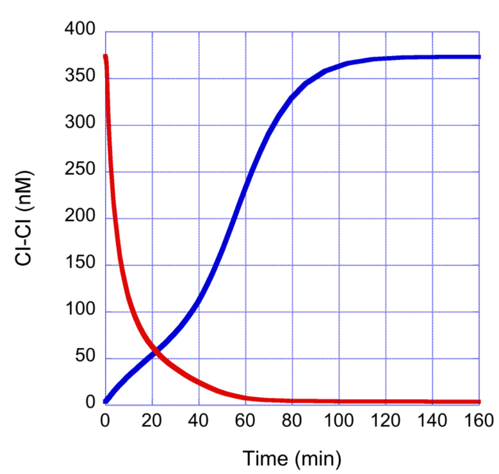
Global Tolerances for the Lysogenic Phenotype

mRNA		Protein	
Parameter	Tolerance	Parameter	Tolerance
γ_M	[2.0,4.2] †	δ_C	[illegible]
$\gamma_{M\,\max}$	[2.4,1.7]	$\delta_{C\,\max}$	[illegible]
K_D	[2.8,1.6]	[illegible]	[82,∞]
K_I	[11,2.4]	a	[5.6,∞]
p	[illegible]	γ_C	[1.6,1.7]
n	[illegible]	γ_D	[1700,2.8]
[illegible]	[1.7,1.6]	β_D	[3.4,1900]
		δ_D	[11,1.6]

Analytical Results

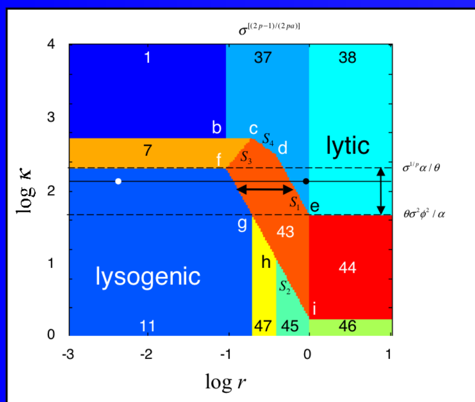
* [fold decrease,fold increase] Coelho et al (2009)

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



$7 \text{ X } \tau_{1/2}$

Implications

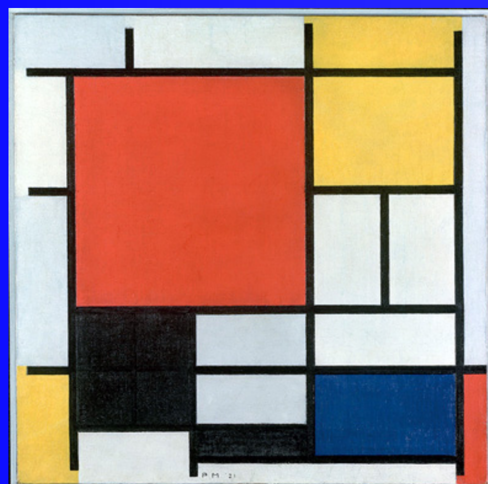


- 15-D parameter space compressed to 2-D design space
- Predicted behavior in each region
  - Lytic
  - Lysogenic
  - Hysteretic
- Two types of pathology
  - Failure to cycle between states
  - Inappropriate switching
- Remarkable asymmetry in switching times favoring induction
- Global tolerance to parameter variation for the lysogen
- Experimental examples of Global tolerance (Little et al.)

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 changes in phenotype
  - Facilitates identification of *system design principles*
- Capable of computer automation

Every true artist has been inspired more by the beauty of lines and color and the relationships between them than by the concrete subject of the picture. -- Piet Mondrian (1921)



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