Neuro-Fuzzy-Chao-Criti-Plexity
in
Scale-free Bio-systems

A HOT 50 years in Biological Design

Robust Yet Fragile Egos in Systems Biology

An autobiography

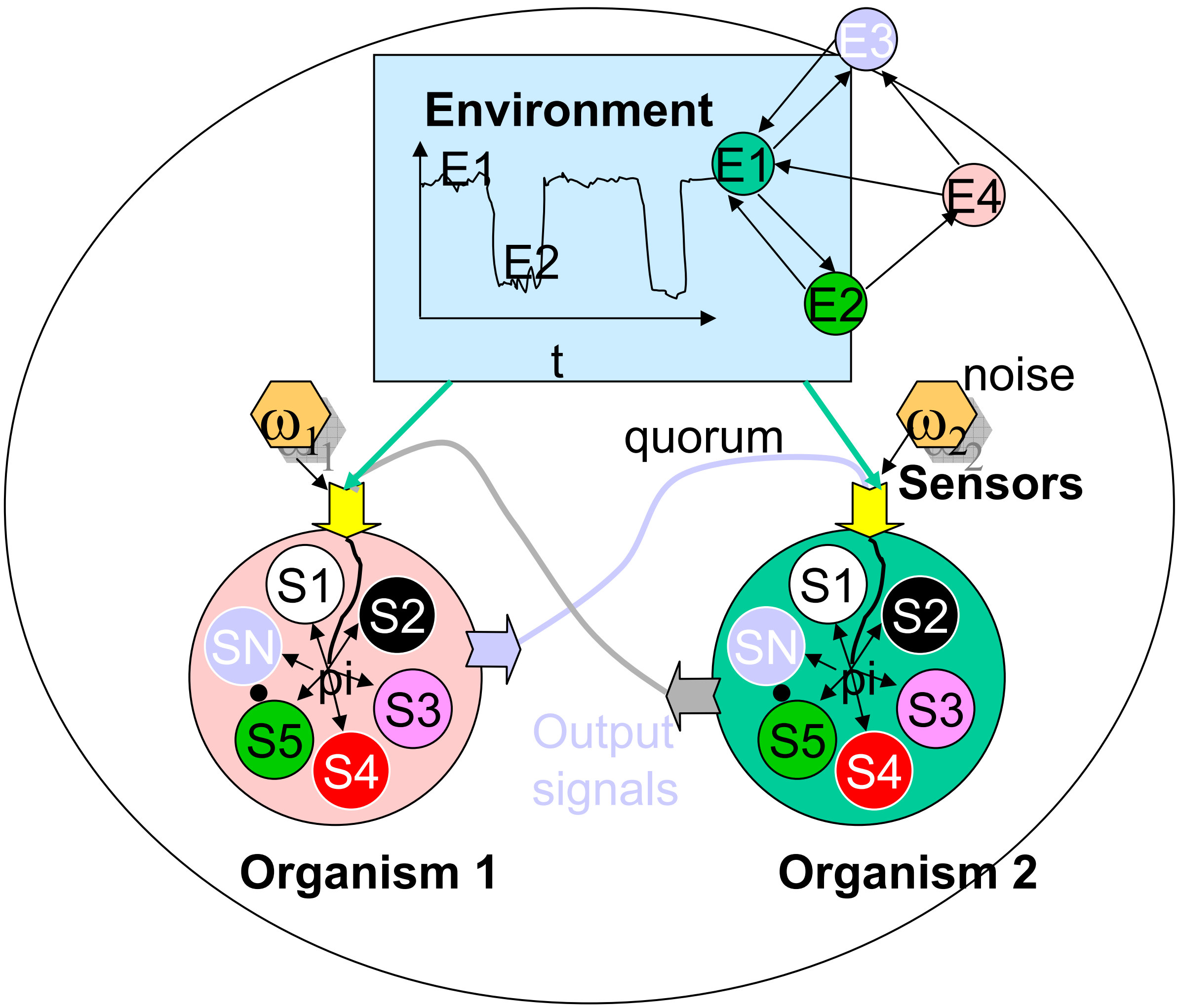
Design and Diversity in Cellular Networks

Agent Smith
The Matrix

I'd like to share a revelation that I've had during my time here. It came to me when I tried to classify your species. I realized that you're not actually mammals. Every mammal on this planet instinctively develops a natural equilibrium with the surrounding environment, but you humans do not.

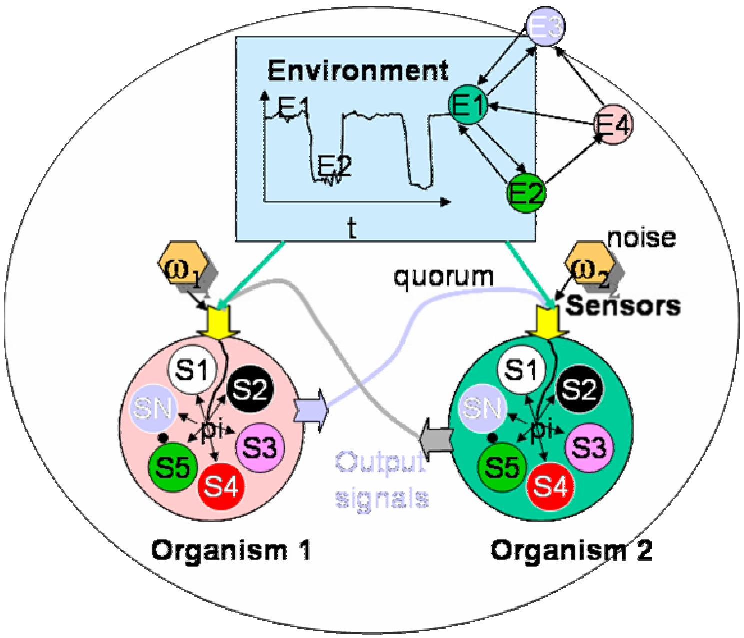
You move to an area, and you multiply, and multiply, until every natural resource is consumed. The only way you can survive is to spread to another area. There is another organism on this planet that follows the same pattern. A virus. Human beings are a disease, a cancer of this planet, you are a plague, and we are the cure.

Everyone’s a virus



It’s a virus eat virus world out there.

Everyone's a virus

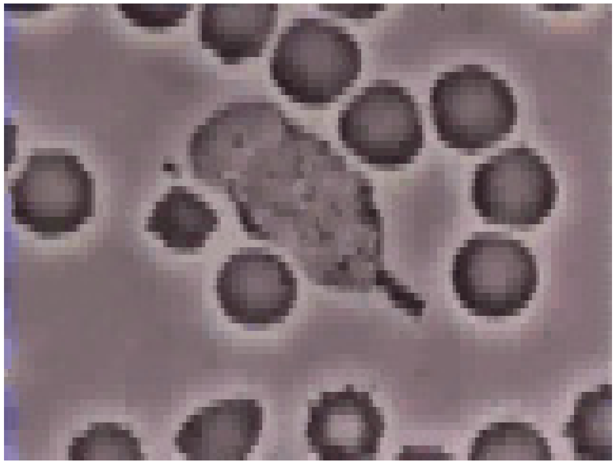


Evolution selects for strategies that win some environmental game played against nature and other organisms.

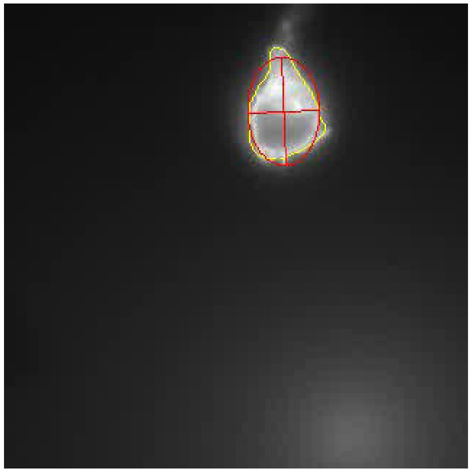
It's a virus eat virus world out there.

Immune cells

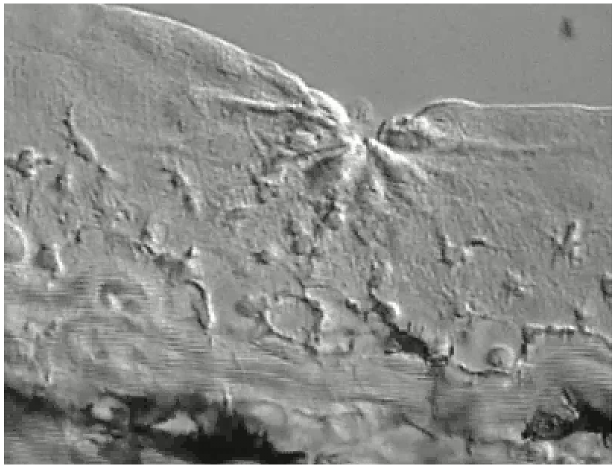
- They perform amazing engineering feats under the control of complex cellular networks



Stossel



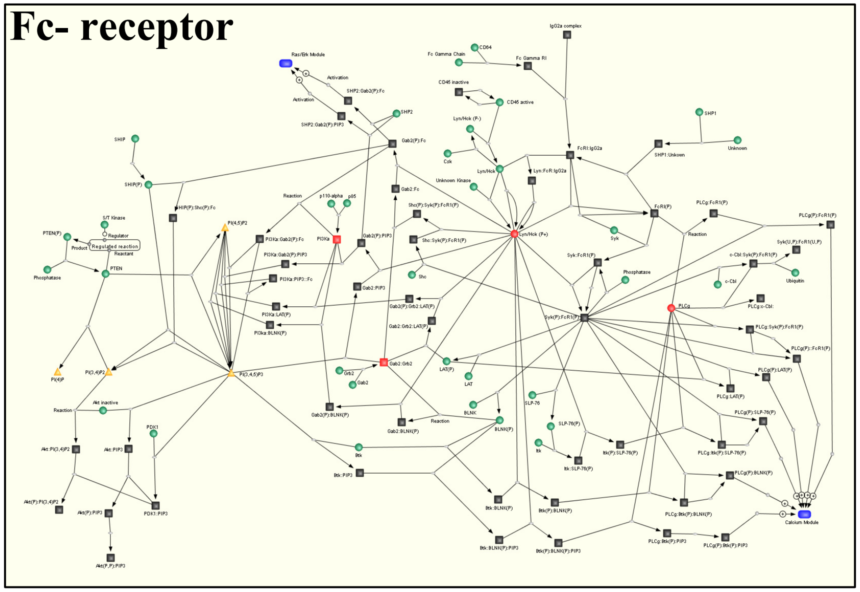
Onsum, Arkin, UCB

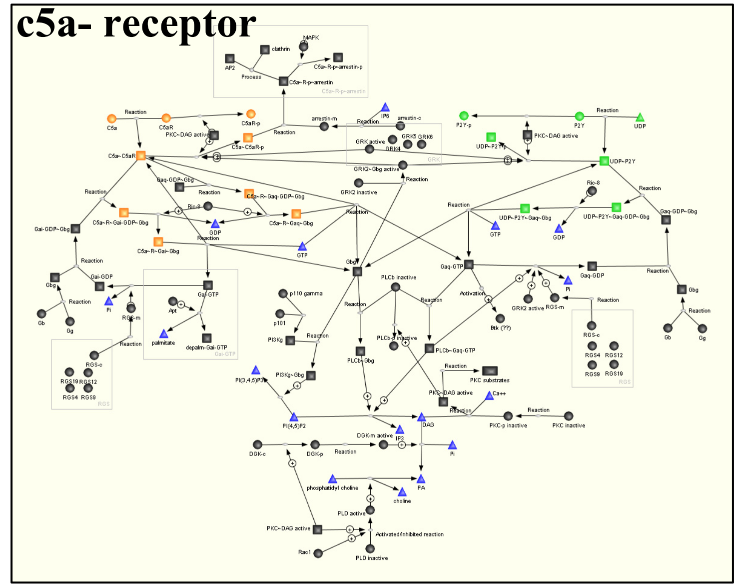


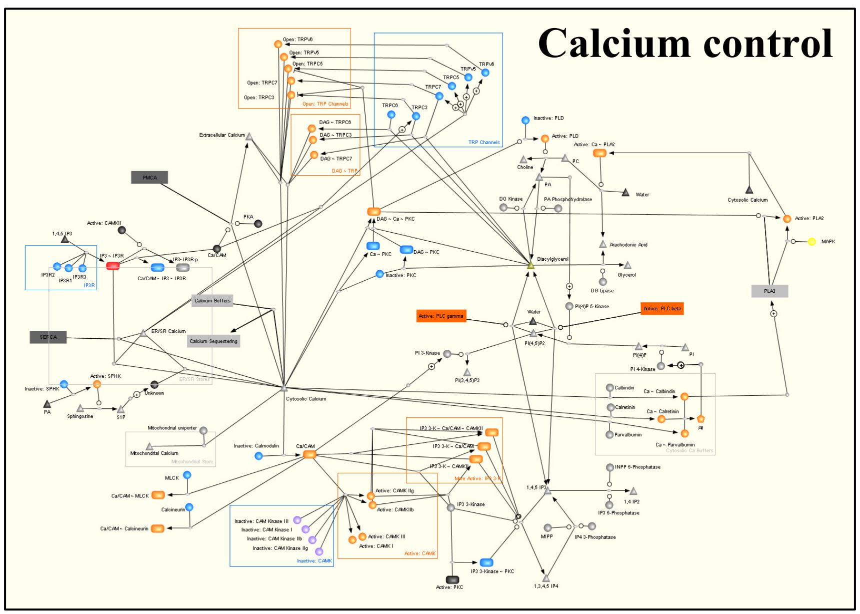
Mione, Redd, UCL

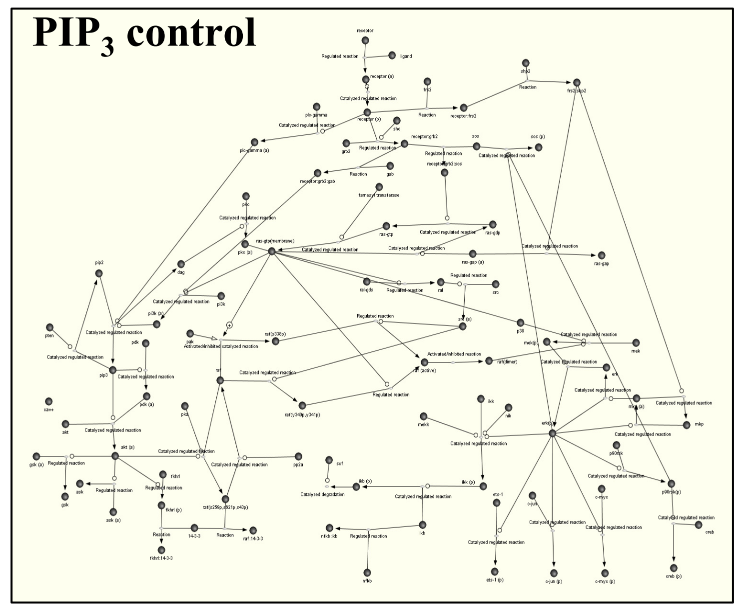
~1/50 of the known macrophage chemotaxis network

Courtesy of AfCS

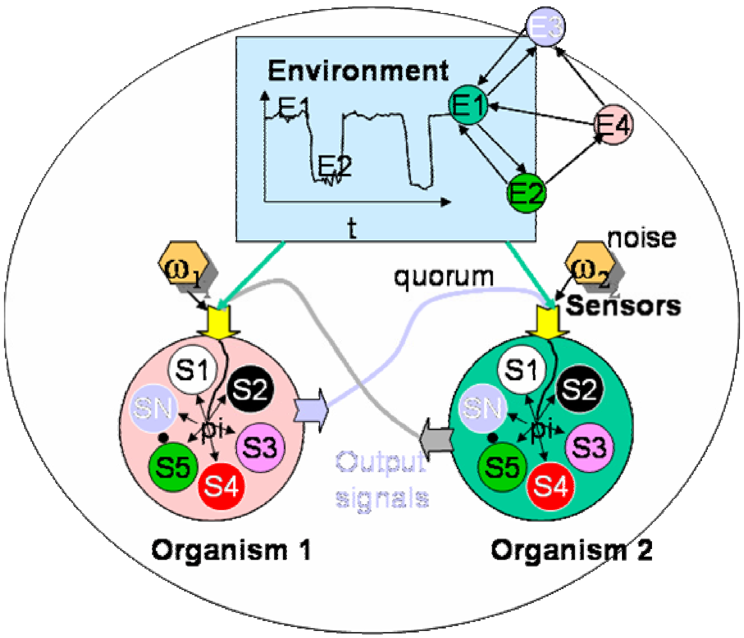








Everyone's a virus

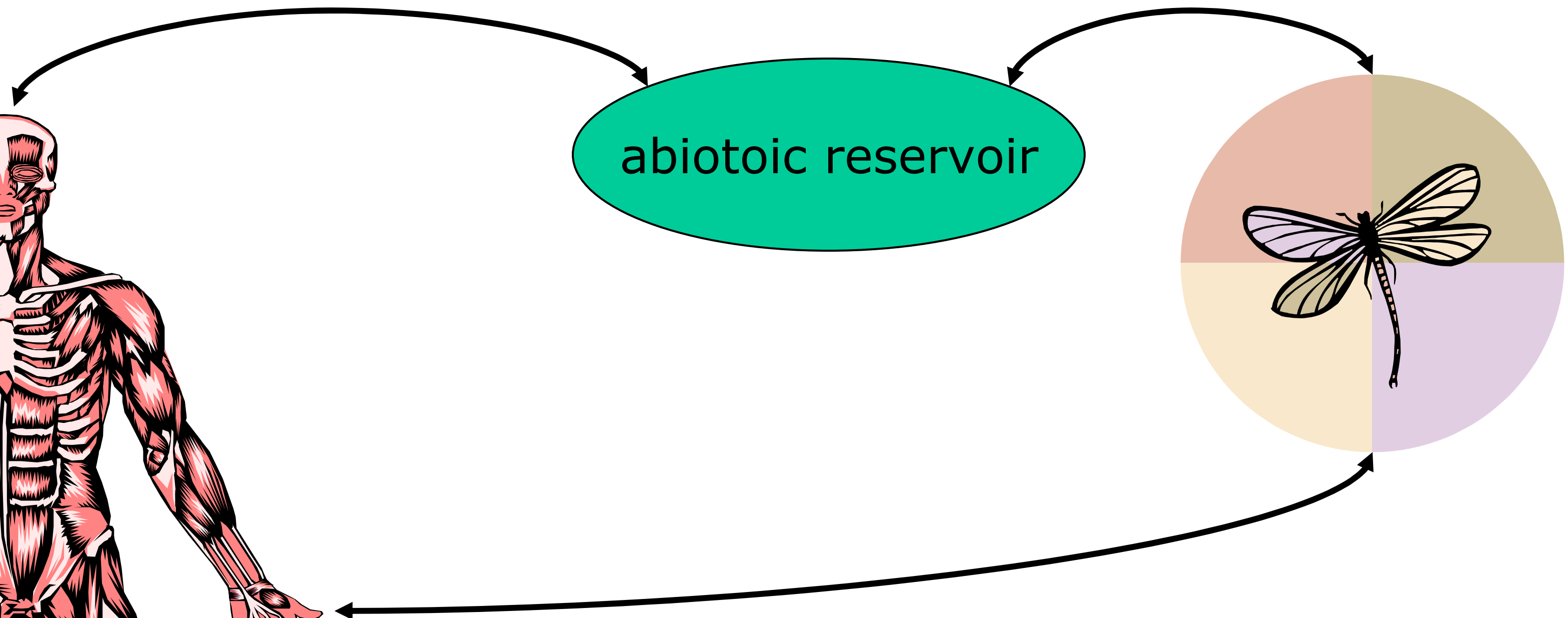


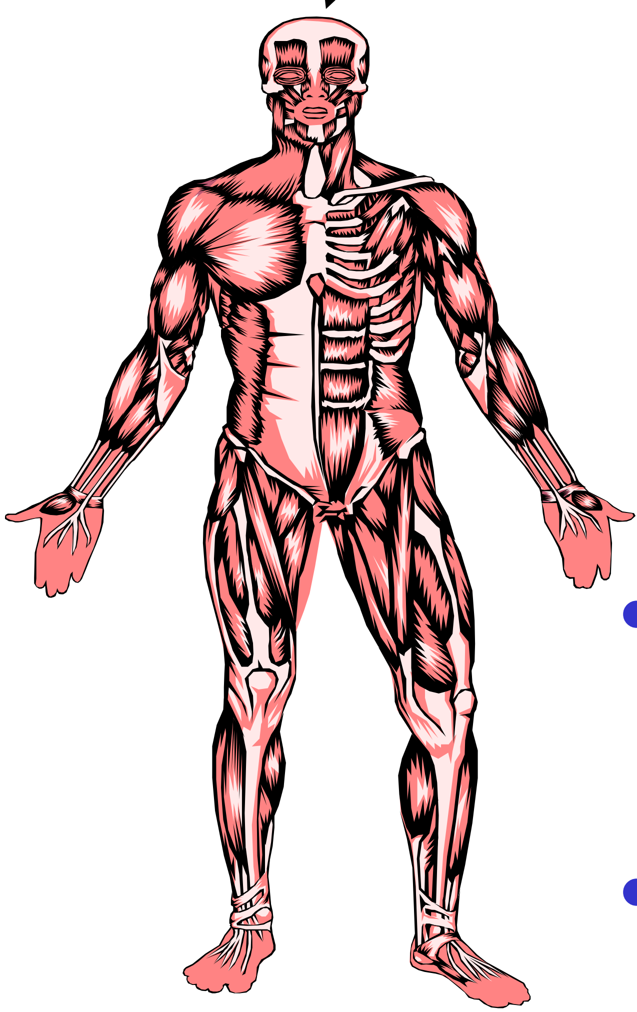
Evolution selects for strategies that win some environmental game played against nature and other organisms.

Ultimately, we want to engineer biological systems that perform a designed function and NO MORE.

It's a virus eat virus world out there.

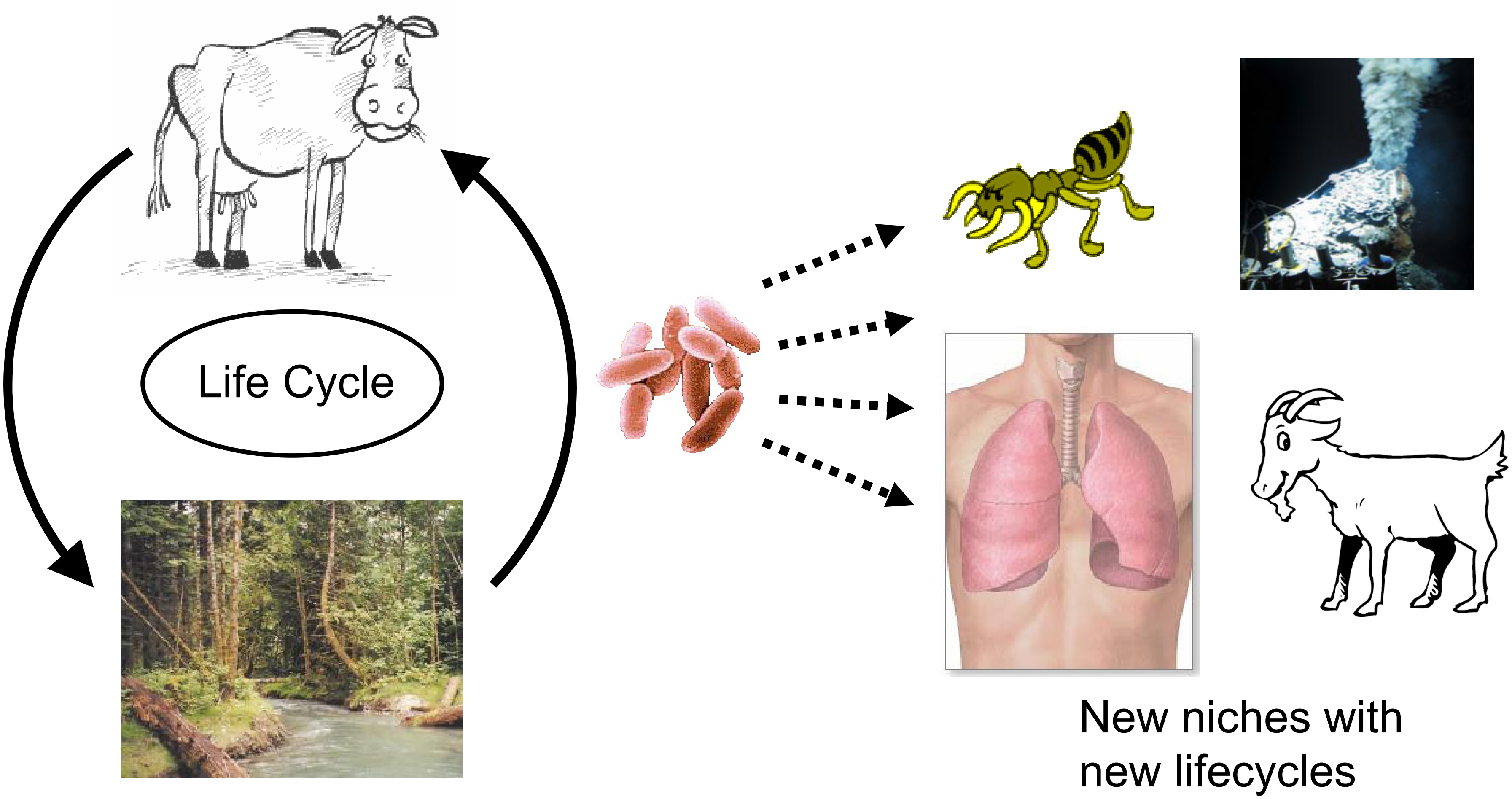
Niches are Dynamic





- Characteristic times may be spent in each environment.
- Environments themselves are variable.

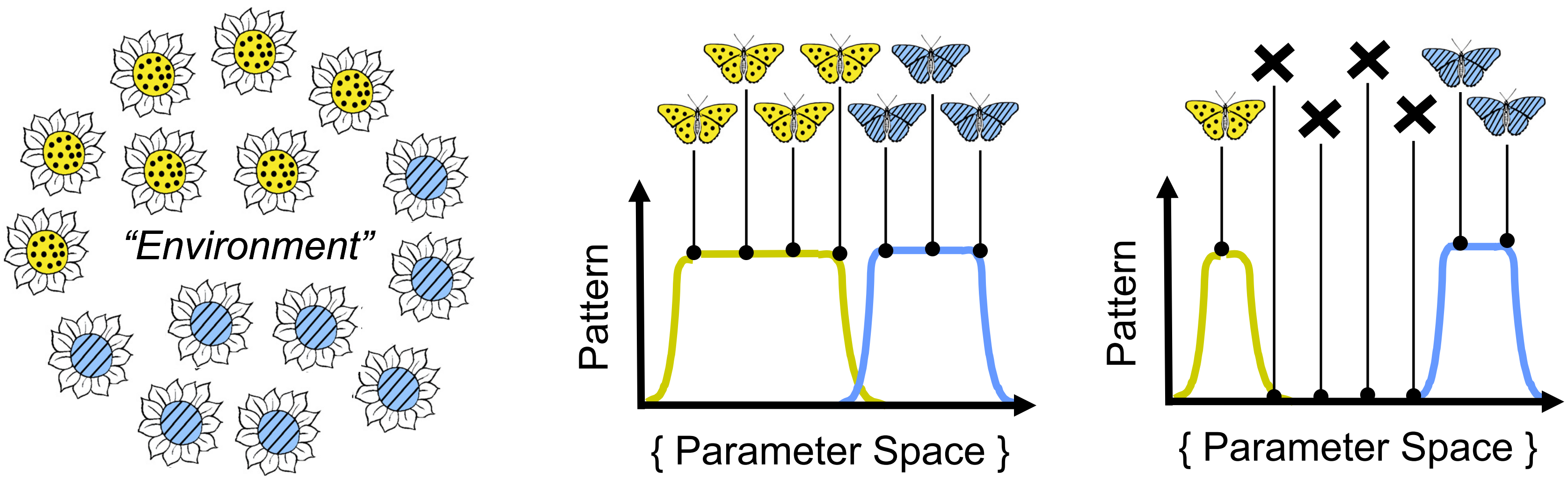
Adaptability vs. Evolvability



- Adaptability: Adjustment on the time scale of the life cycle of the organism
- Evolvability: Capacity for genetic changes to invade new life cycles

Evolvability

- In a dynamic environment, the lineage that adapts first, wins
- Fewer mutations means faster evolution
- Are some biosystems constructed to minimize the mutations required to find improvements?



- Modularity
- Robustness / Neutral drift improves functional sampling
- Shape of functionality in parameter space
- Minimize null regions in parameter space (entropy of multiple mutations)

Systems and Synthetic Biology

- **Systems biology seeks to uncover the design and control principles of cellular systems through**
  - Biophysical characterization of macromolecules and other cellular structures
  - Comparative genomic analysis
  - Functional genomic and high-throughput phenotyping of cellular systems
  - Mathematical modeling of regulatory networks and interacting cell populations.

- **Synthetic biology seeks to develop new designs in the biological substrate for biotechnological, medical, and material science.**
  - Founded on the understanding garnered from **systems biology**
  - New modalities for genetic engineering and directed evolution
  - Scaling towards programmable biomaterials.

Systems biology is necessary

- Because of the highly interconnected nature of cellular networks
- Because it is the best way to understand what is controllable and what is not in pathway dynamics
- Because it discovers what designs evolution has arrived at to solve cellular engineering problems that we emulate in our own designs.

Synthetic biology

- There have been an number of impressive feats in design of cells that foreshadow a true revolution in rational cellular engineering!
- Our ability to manufacture new molecules and proteins with new and useful functional groups is rapidly outstripping our ability to predictively engineer new behavior into cells or control natural behavior.
- Cells are sensing, actuating, and evolving platforms for our engineering– our additions to them do not operate in isolation.

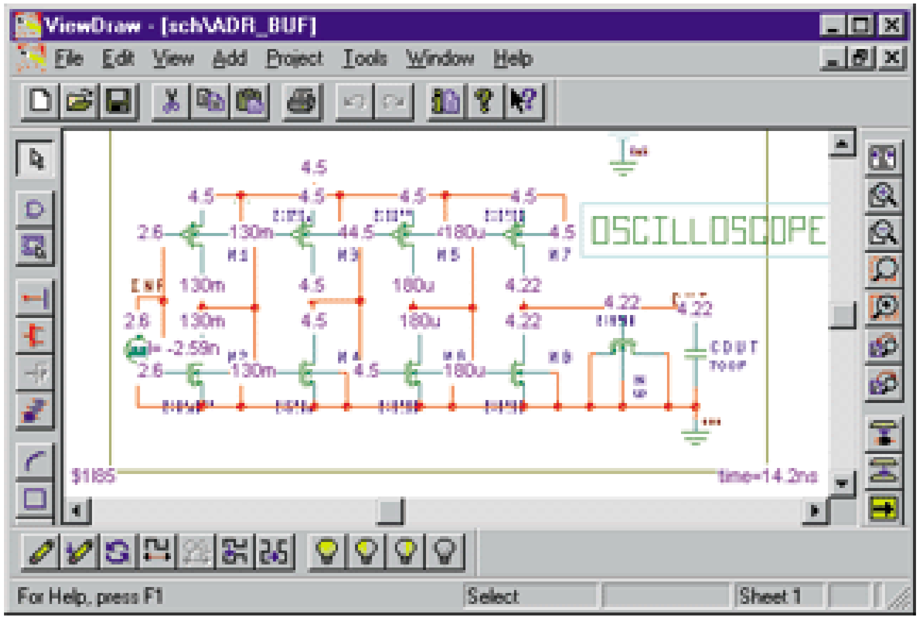
Design in Cellular Networks

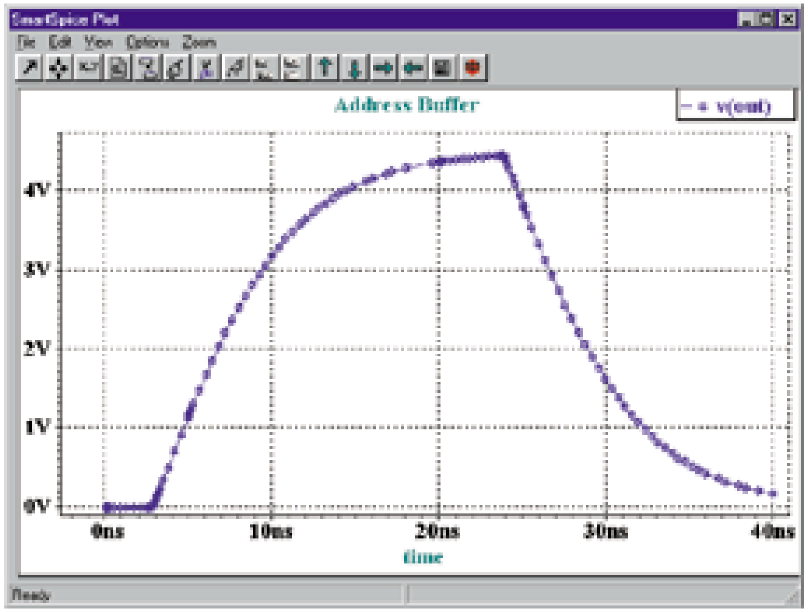
Biological device physics

- Analog, asynchronous, nonlinear, and stochastic
- Evolution creates designs that make robust cellular function despite this
- Unlike in electronics, the theory for biomolecular devices is early at best and measurement technology is only beginning to mature.

SPICE Circuit Simulator

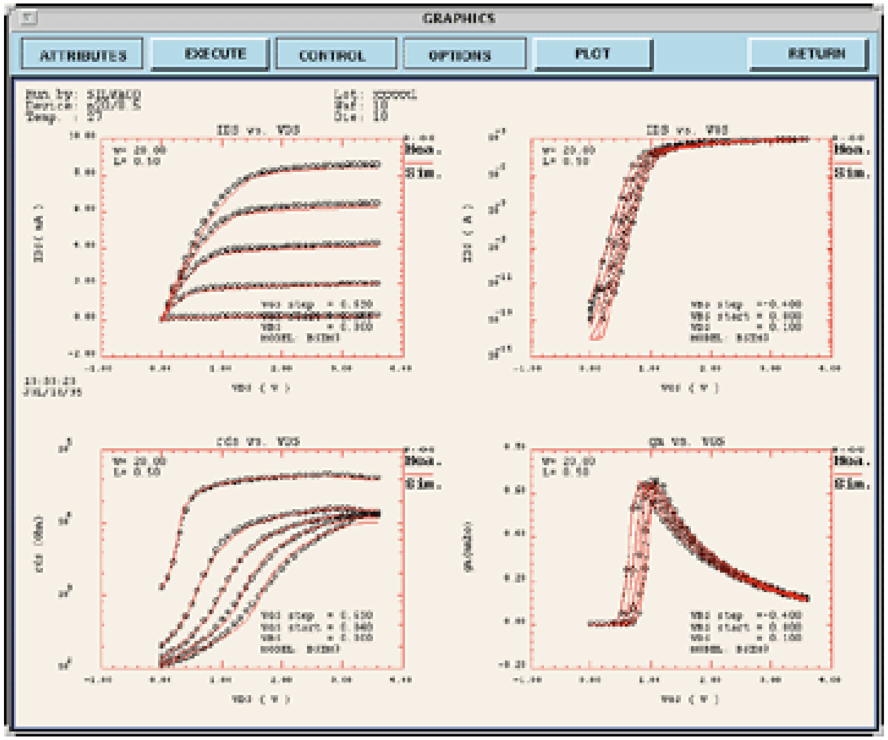
- SPICE is a widely used circuit analysis package which allows the designer to connect electronic devices into a circuit
  - and predicts the response of the circuit under specified conditions
- SPICE is a circuit *simulator*
  - it applies circuit analysis equations to the designed circuit to calculate currents and voltages as a function of time
  - for any condition, it may require a lot of calculations to reach a final answer where all the values are internally consistent





- But how does SPICE ‘know’ how a transistor behaves?

- Microelectronic manufacturing ‘fabs’ will measure thousands of devices in order to get accurate SPICE models
- A widely used SPICE model for transistors is BSIM 3.3
- The better the theoretical framework, the more generally applicable will be the results
  - and the model can be refined

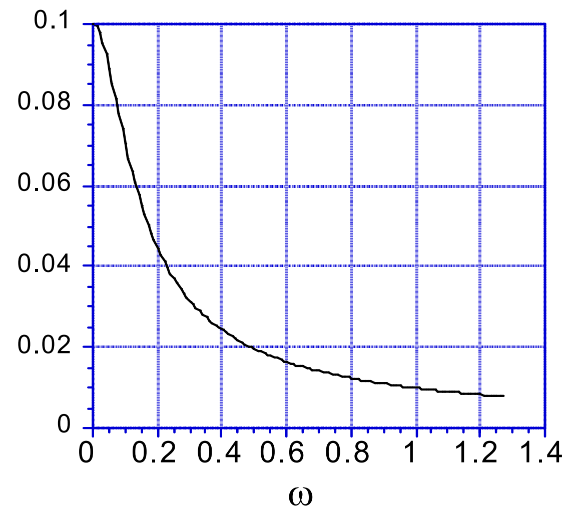


Chemical Frequency Filtering

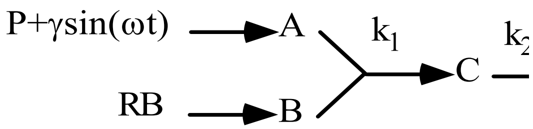
First Order Reaction

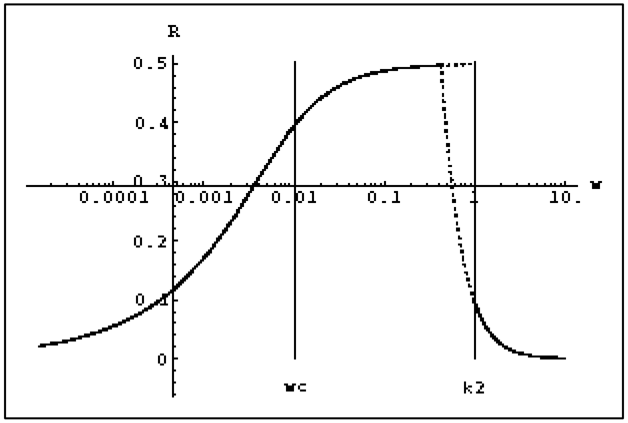
$$\gamma(1+\sin(\omega t)) \longrightarrow A \xrightarrow{k}$$

$$\frac{|A|}{|\gamma \sin(\omega t)|} = \frac{k}{(1 + \omega^2/k^2)^{1/2}}$$



Second Order Reaction



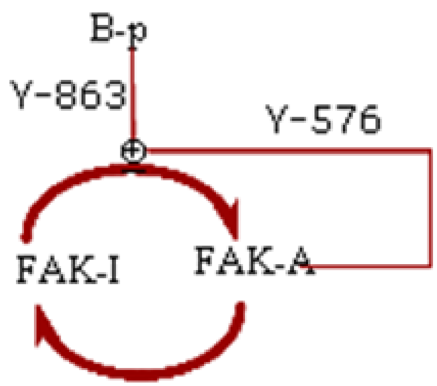


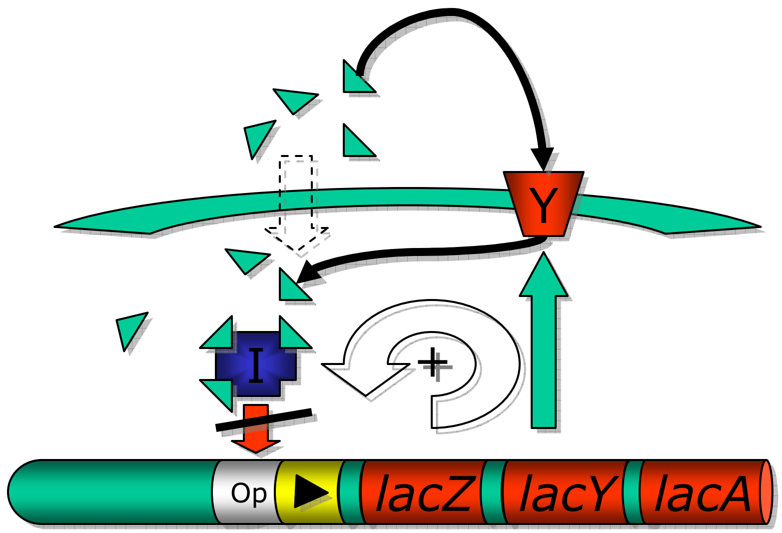
Log(ω)

Equation for amplitude
very complicated

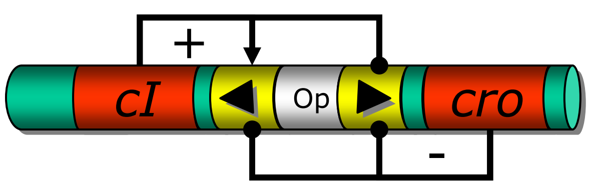
Positive feedback motifs

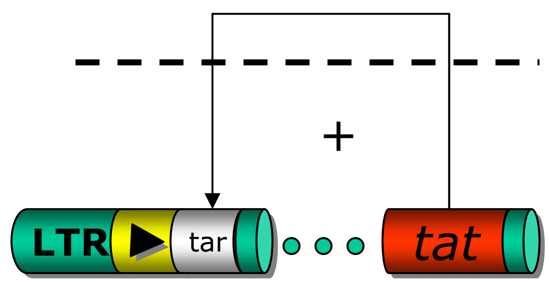
- FAK





- LAC
- Lambda

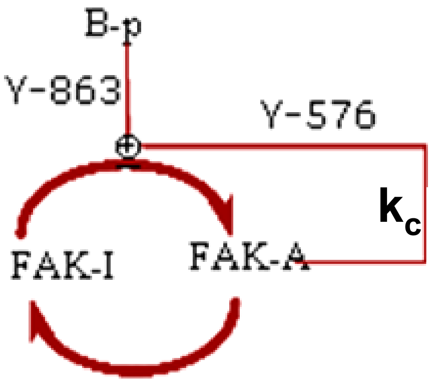


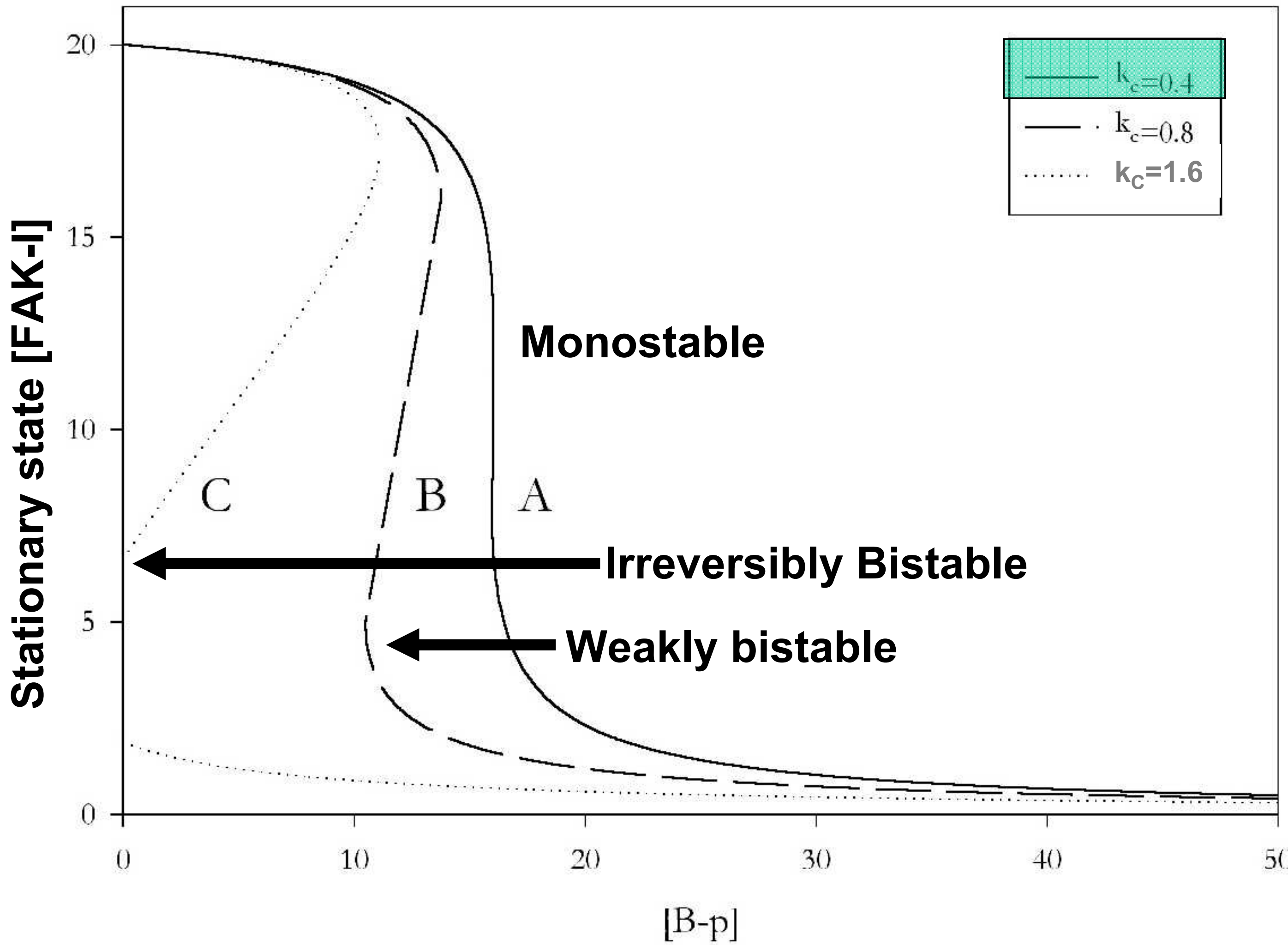


- HIV

Bistability

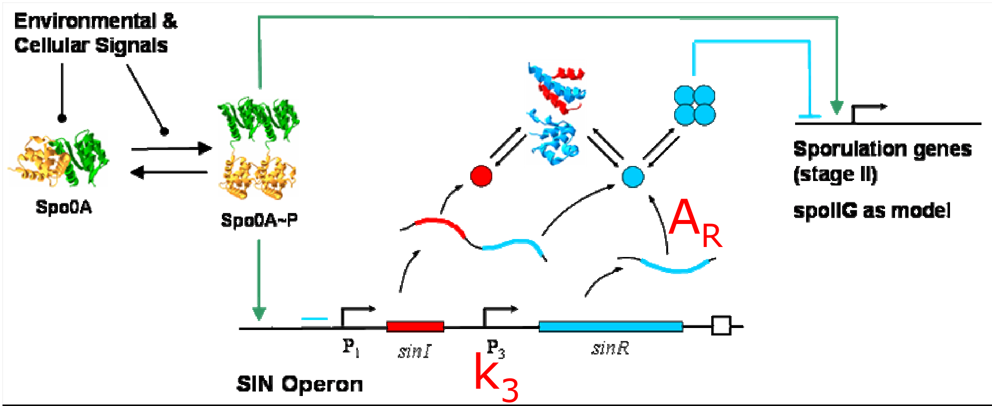
A simple model of the positive feedback

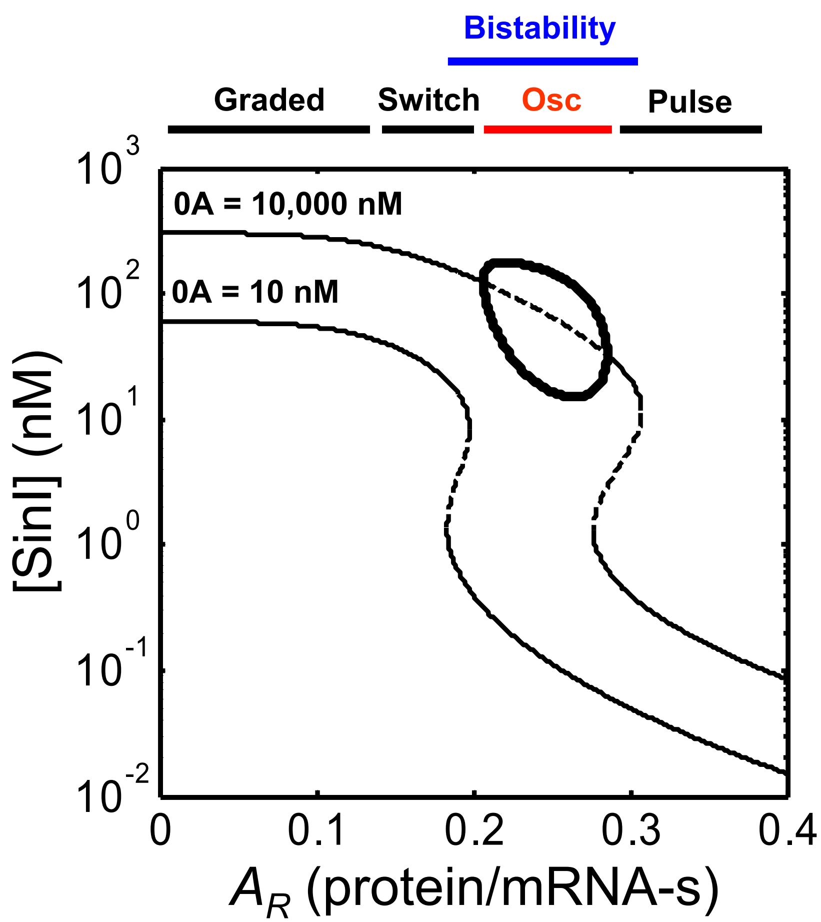


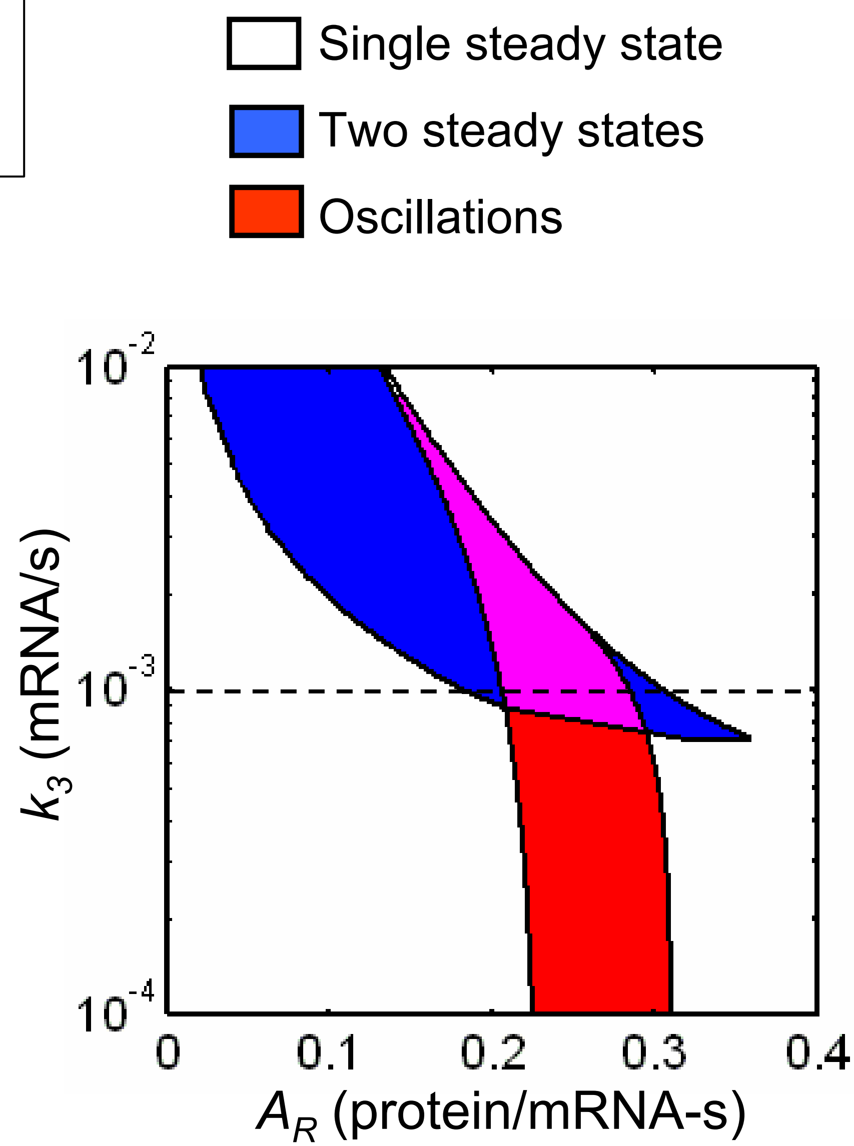


k_c – catalytic constant for the trans-autophosphorylation.

Evolvability?

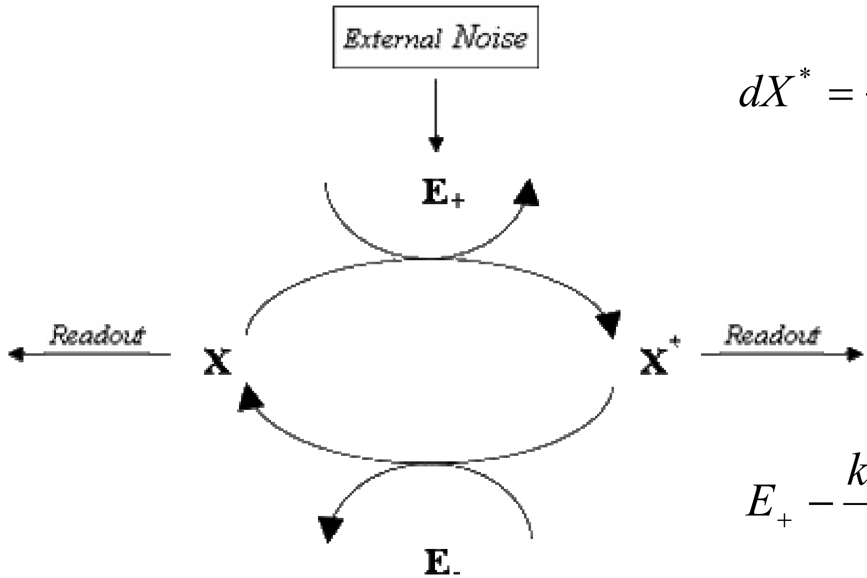






Chris Voigt

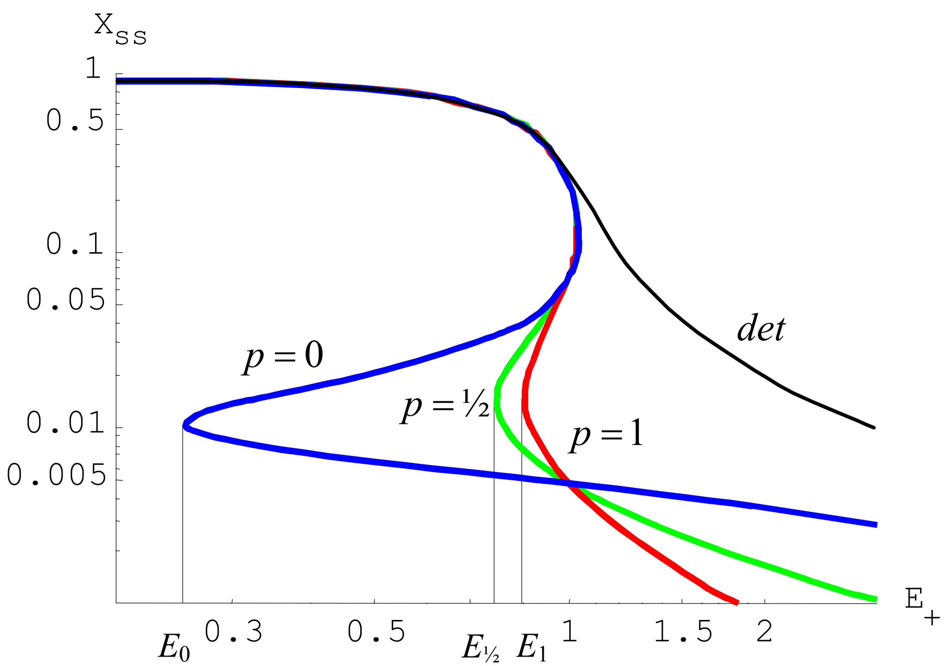
Enzymatic Futile Cycle w/ Noise

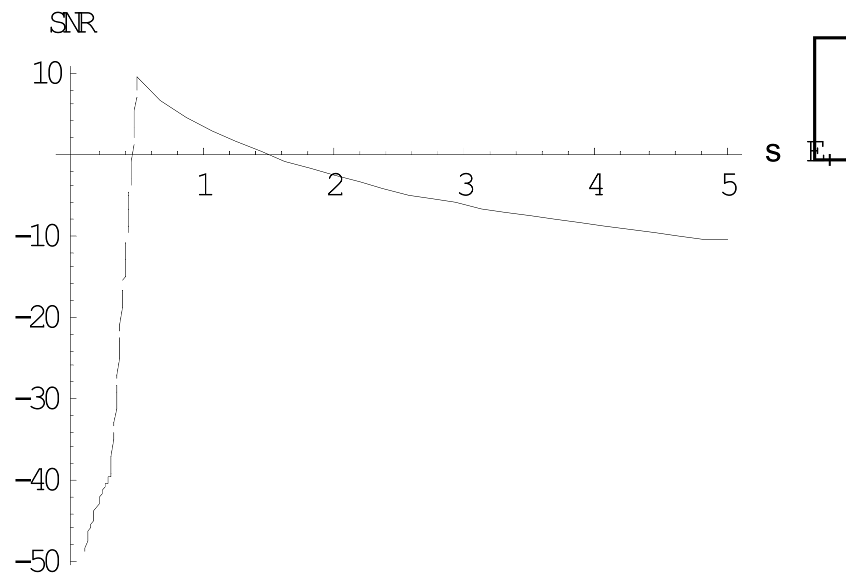


$$dX^* = -dX = \left[\frac{k_+ E_+ X}{K_+ + X} - \frac{k_- E_- X^*}{K_- + X^*}\right] dt + \sigma_+ \frac{k_+ X}{K_+ + X} f(E_+)\, dB_t$$

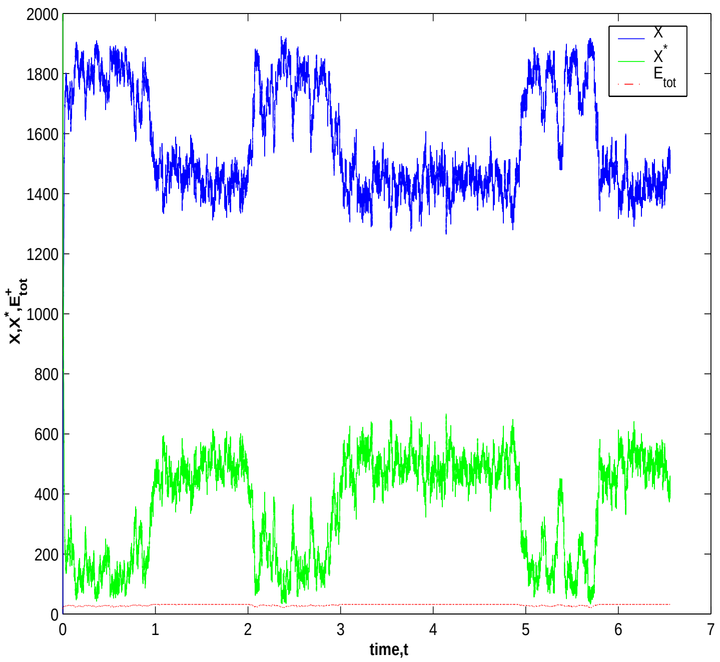
$$f(E_+) = E_+^p$$

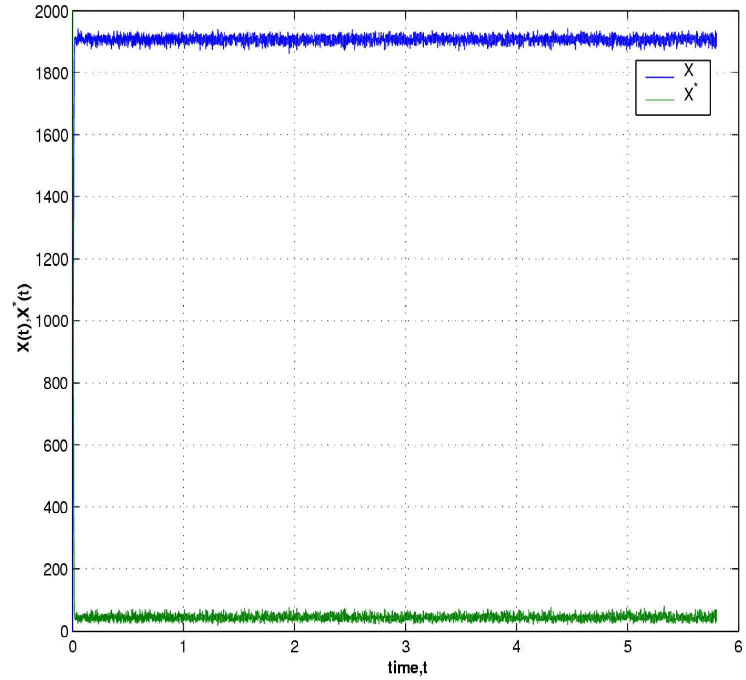
$$E_+ - \frac{k_- E_- (X_0 - X_{ss})(K_+ + X_{ss})}{k_+ X_{ss}(K_- + X_0 - X_{ss})} + \frac{k_+ K_+ \sigma_+^2}{(K_+ + X_{ss})^2} f(E_+)^2 = 0$$

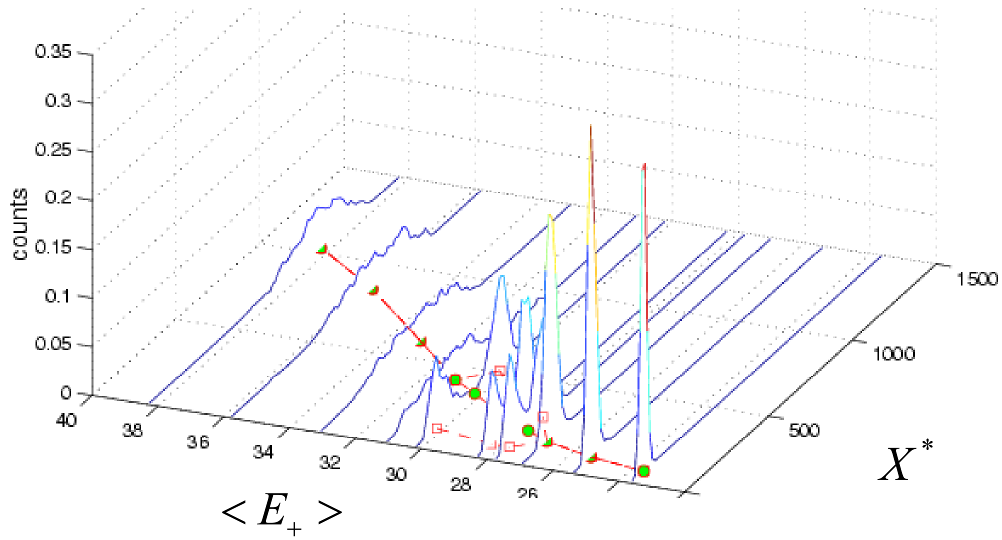


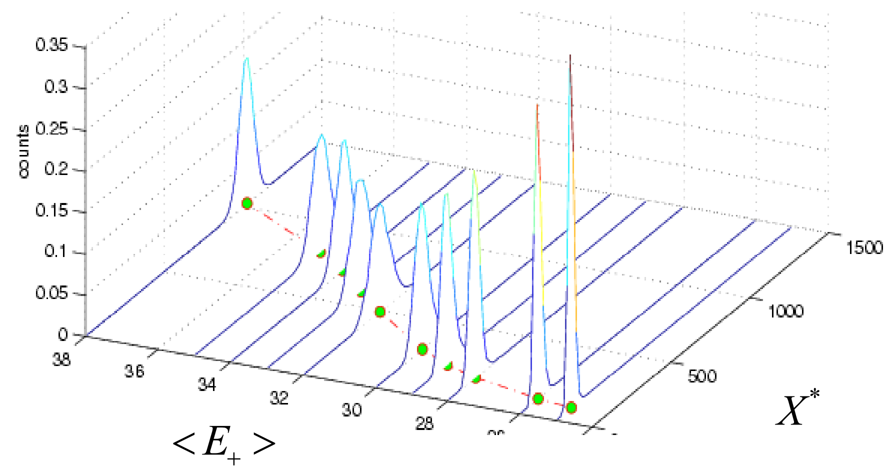


Noise induced Bistability



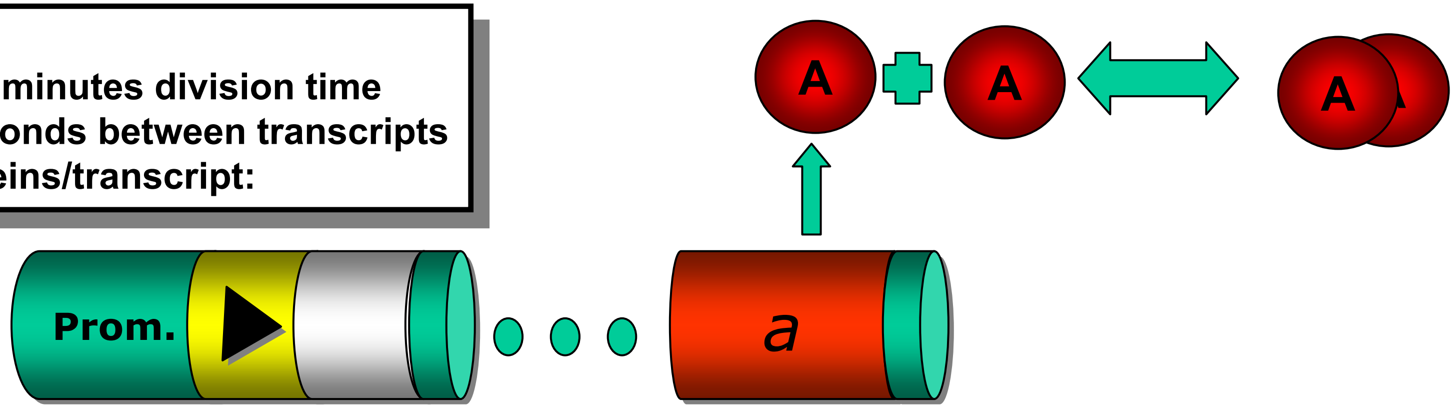


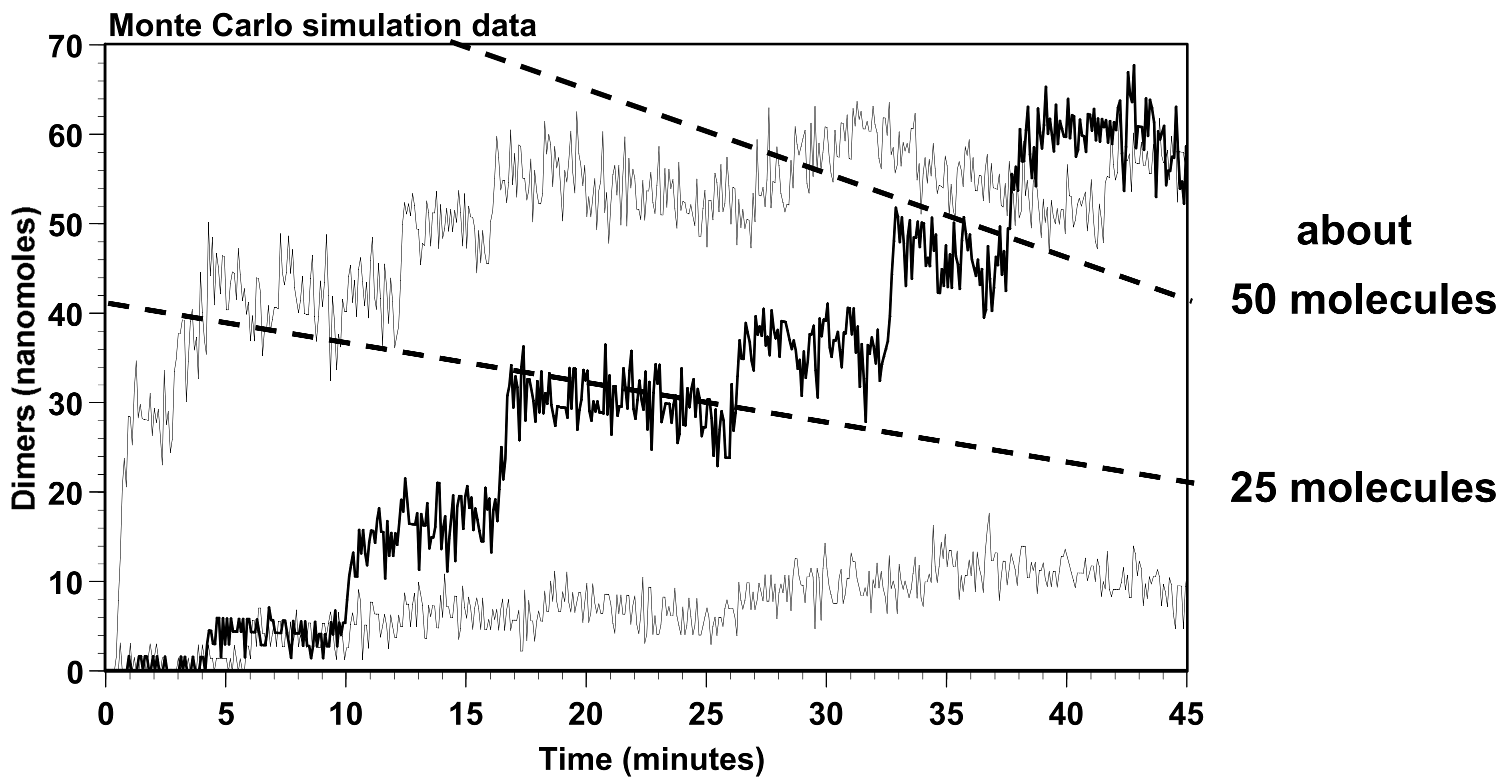




Noise

- One gene
- Growing cell, 45 minutes division time
- Average ~60 seconds between transcripts
- Average 10 proteins/transcript:

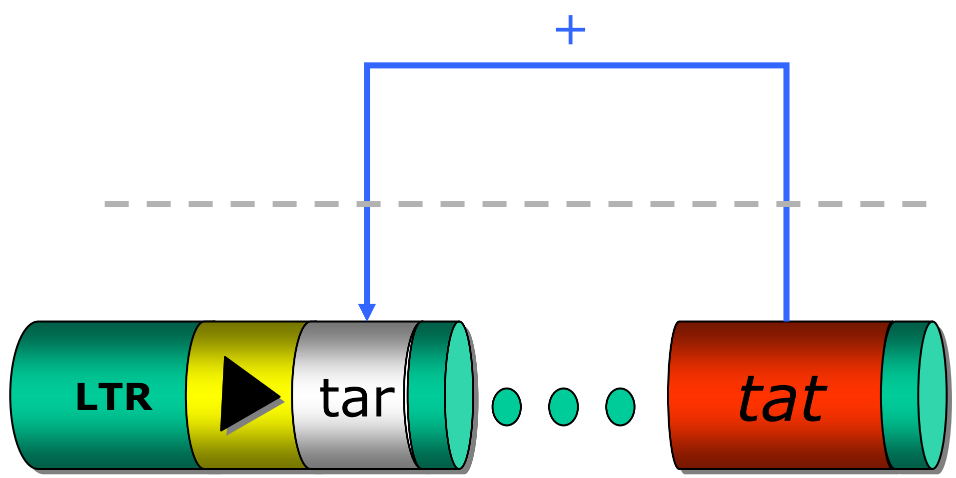




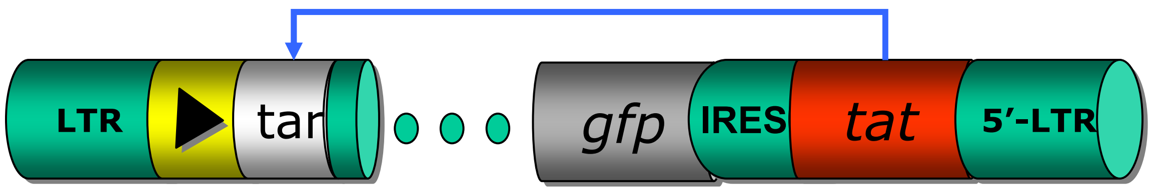
Tat positive-feedback loop

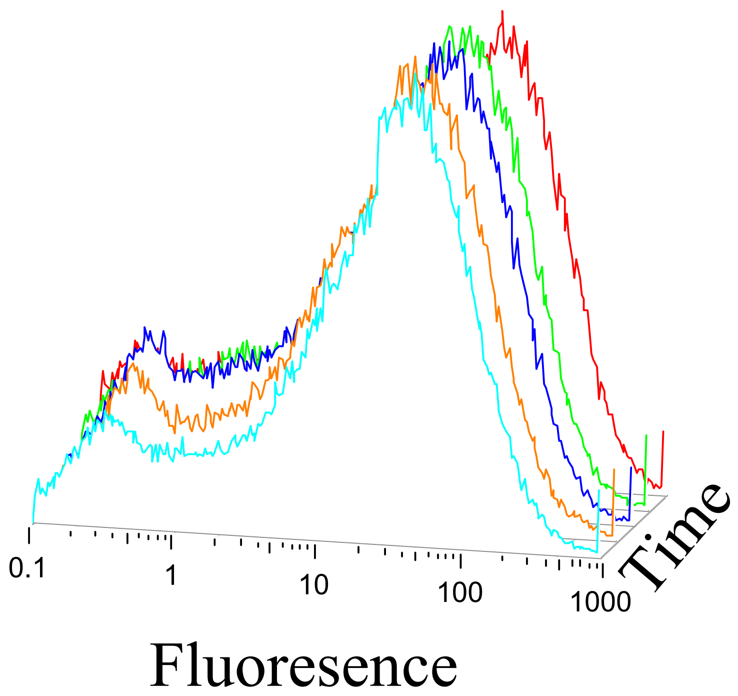
- A standard engineering motif controls, in part, HIV gene expression.

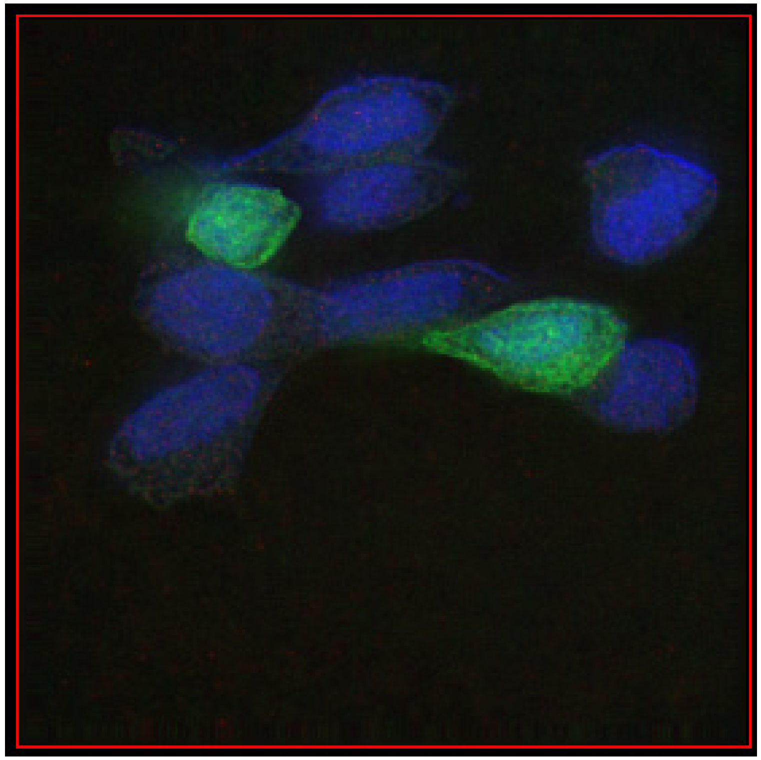
- A positive feedback loop



Viral Bistability





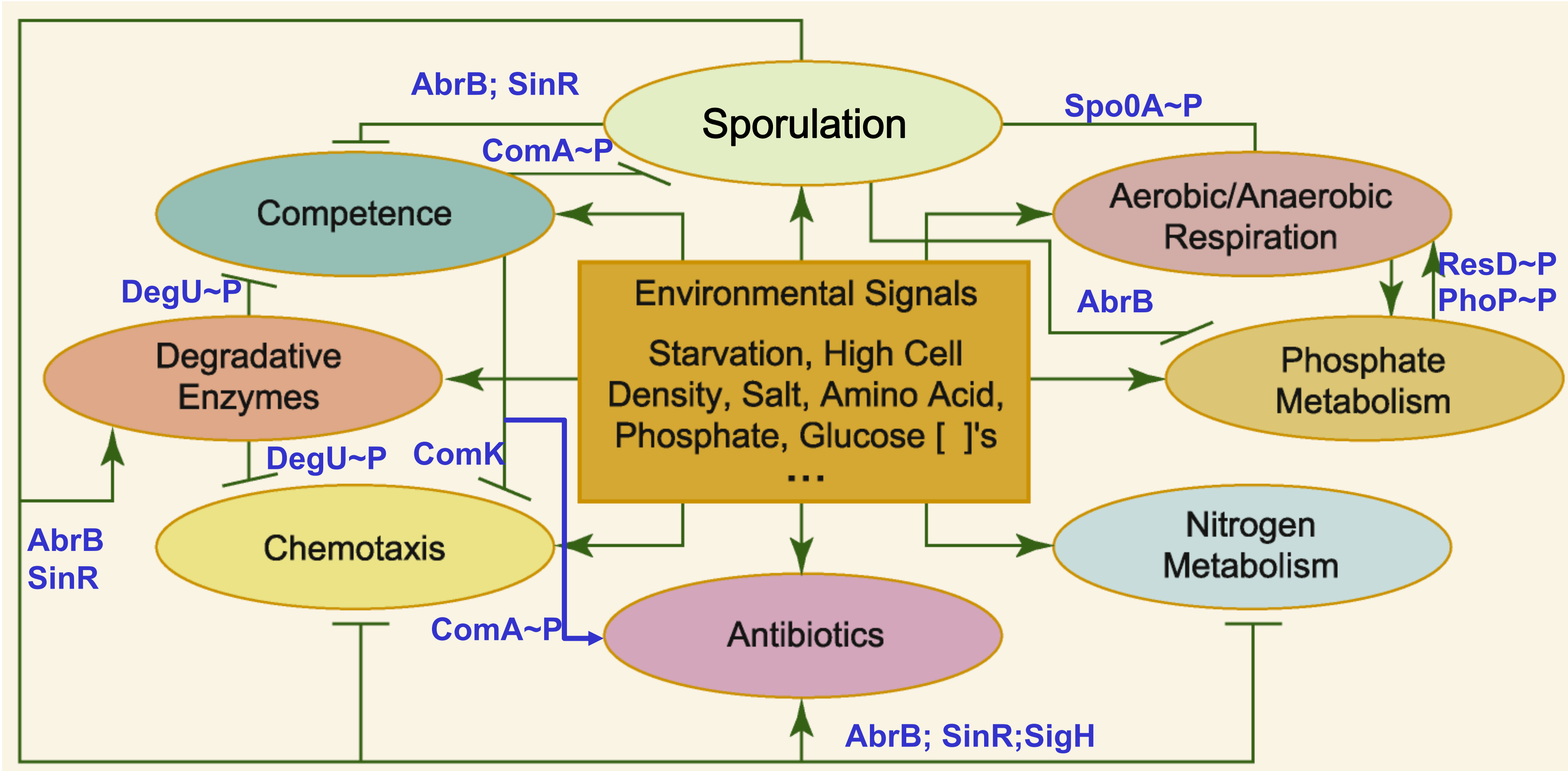


Cells are collections of evolved functions

- Evolution has created a large repository of parts and capabilities who functions and interconnects we are just beginning to understand
- Discovery of new sequence and comparative analysis increasing the rate of confident hypothesis generation about these functions within limits.
- Biophysical characterization lags behind and lacks the "network effect" that sequence and even structure analysis currently enjoys.

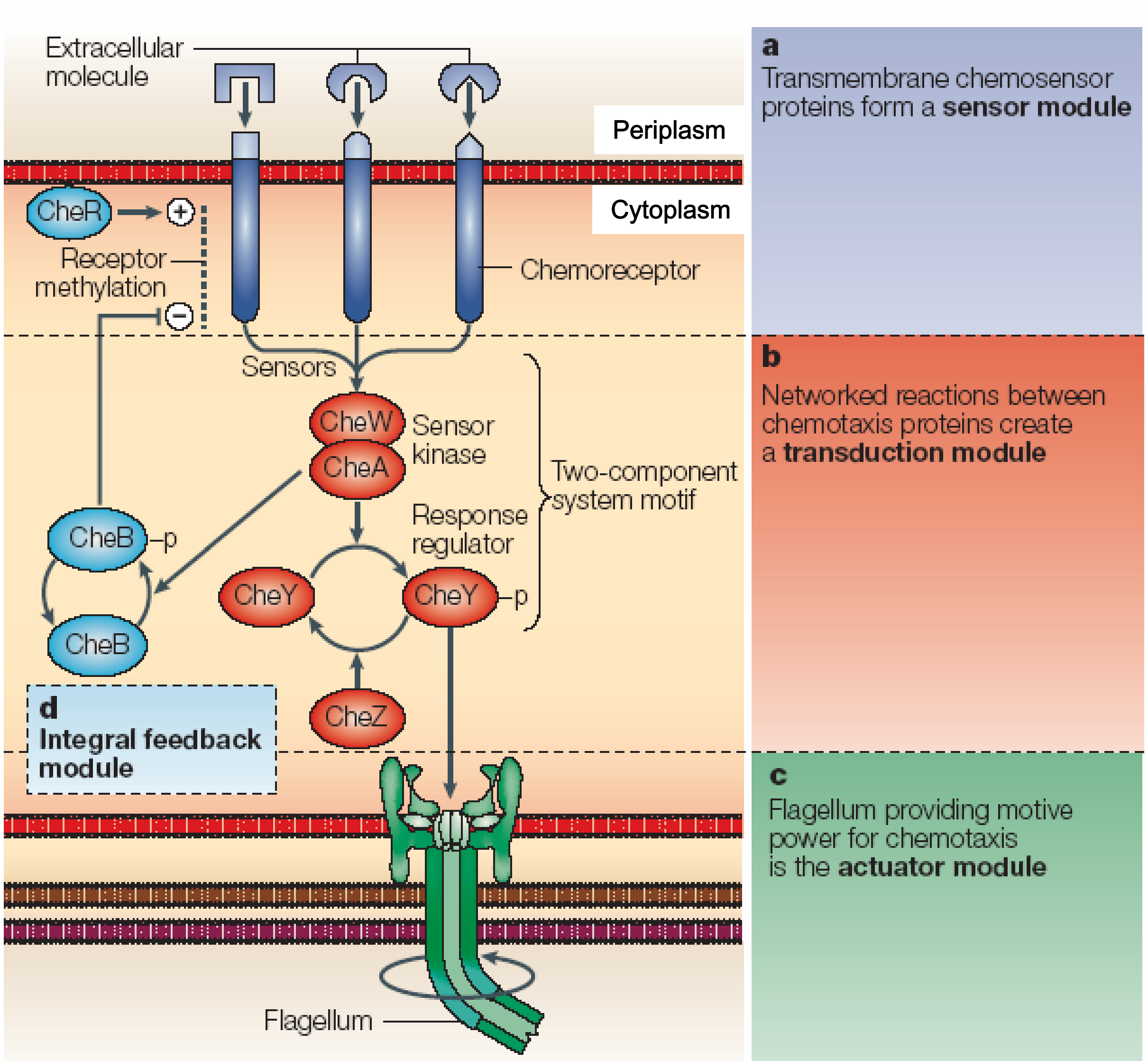
Modules and Systems

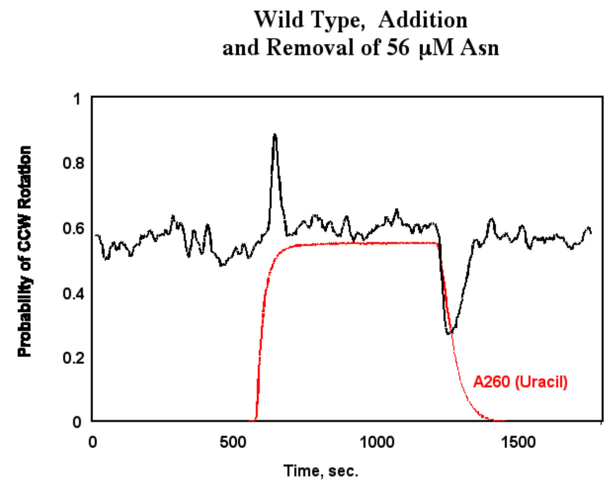
Logic of B.subtilis stress response



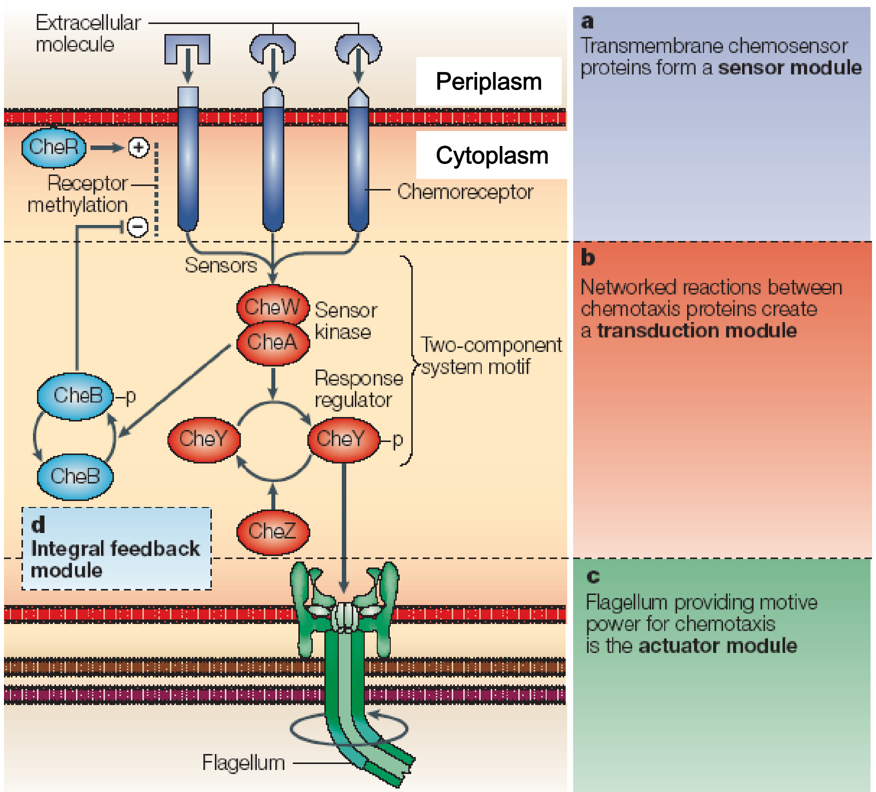
- Network organization has a functional logic.
- There are different levels of abstraction to be found.

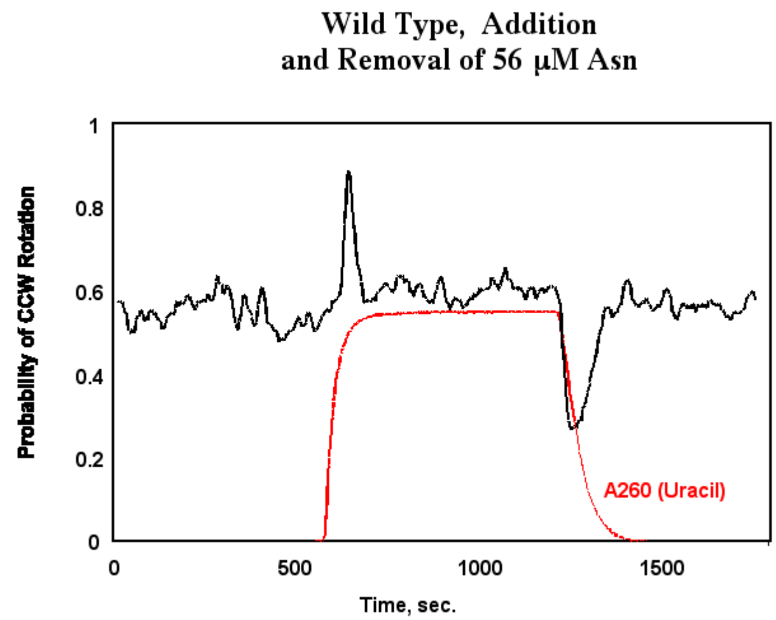
Consider Chemotaxis: E. coli



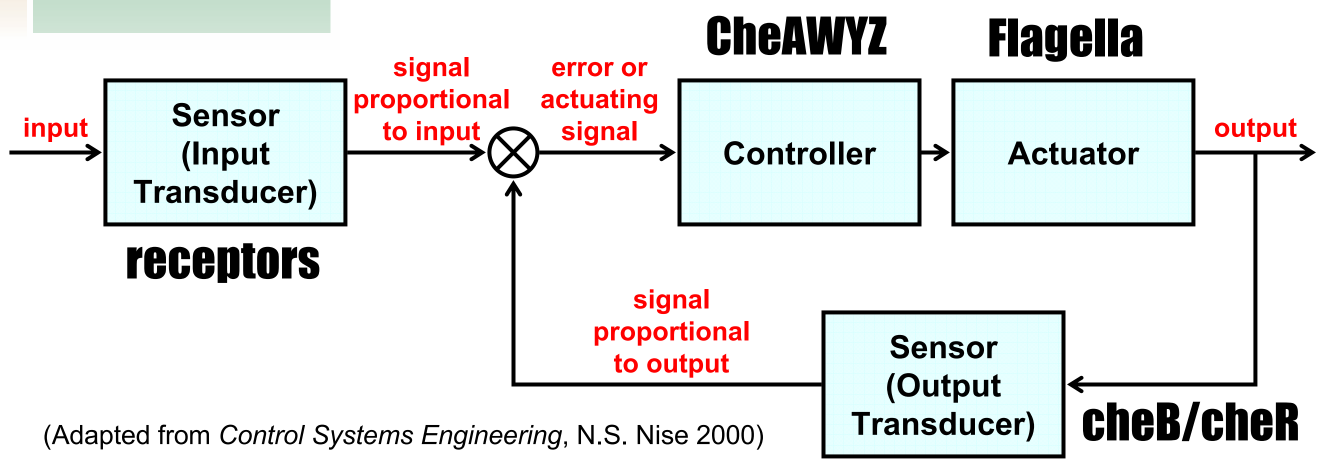


Consider Chemotaxis: E. coli



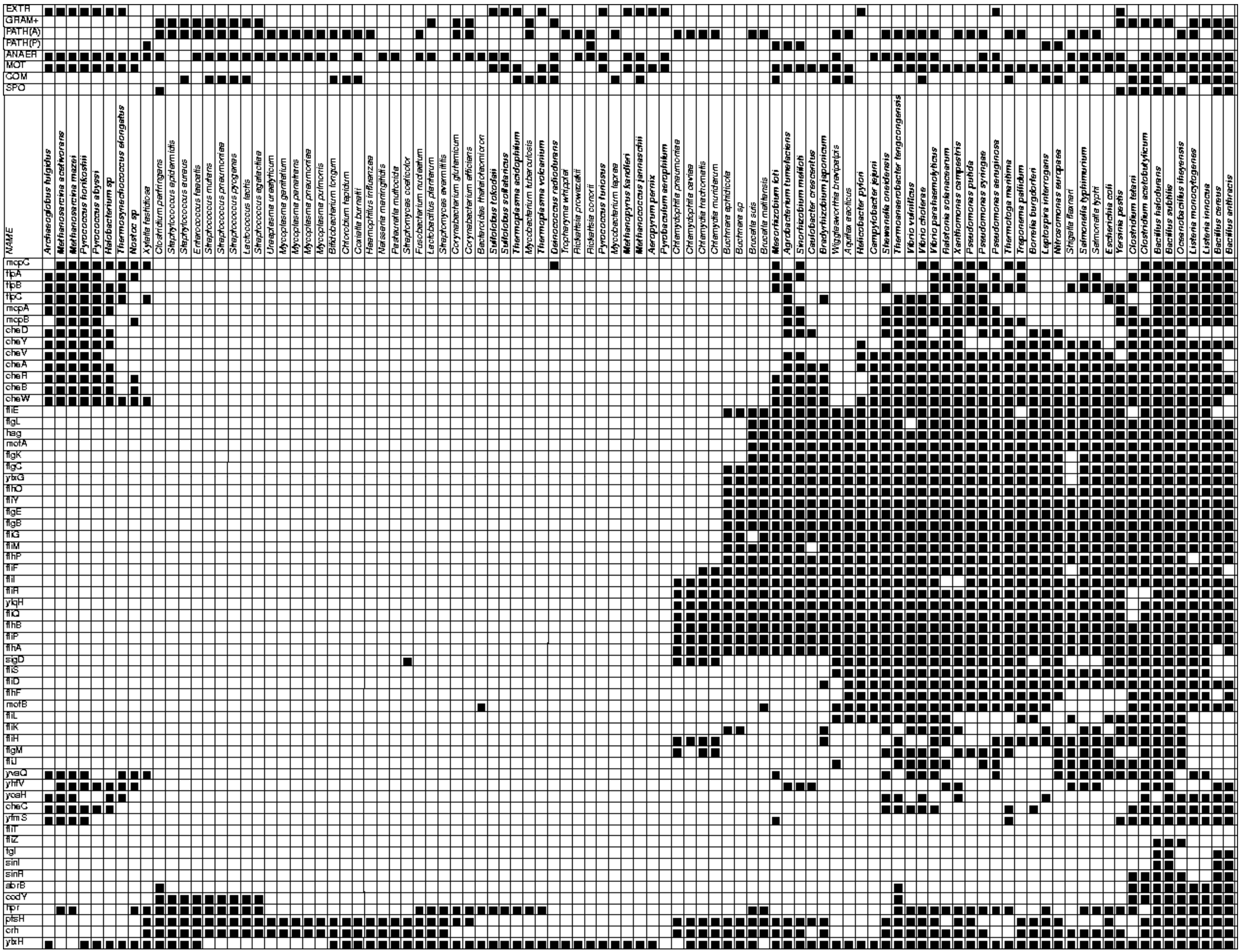


Integral Feedback Controller

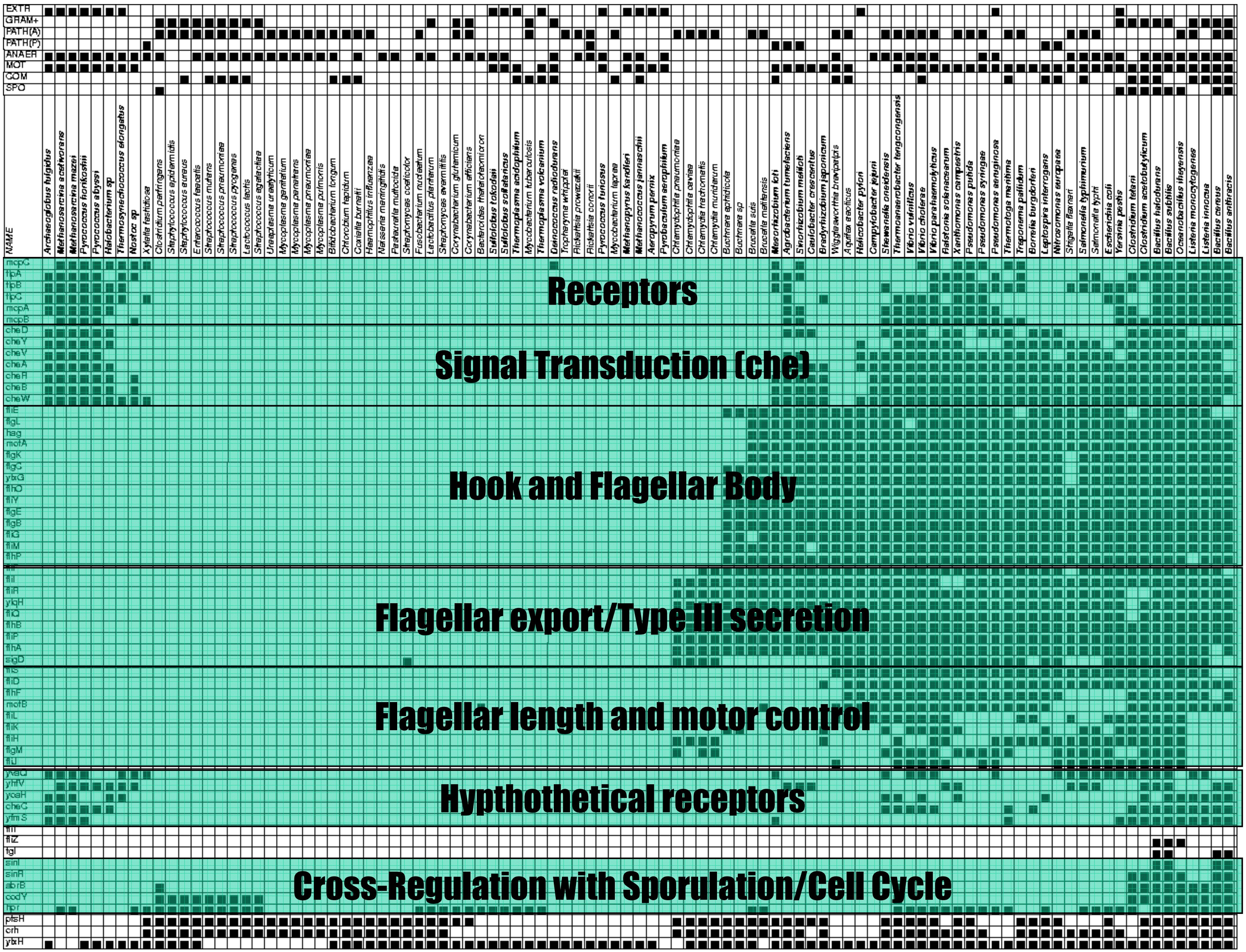


(Adapted from *Control Systems Engineering*, N.S. Nise 2000)

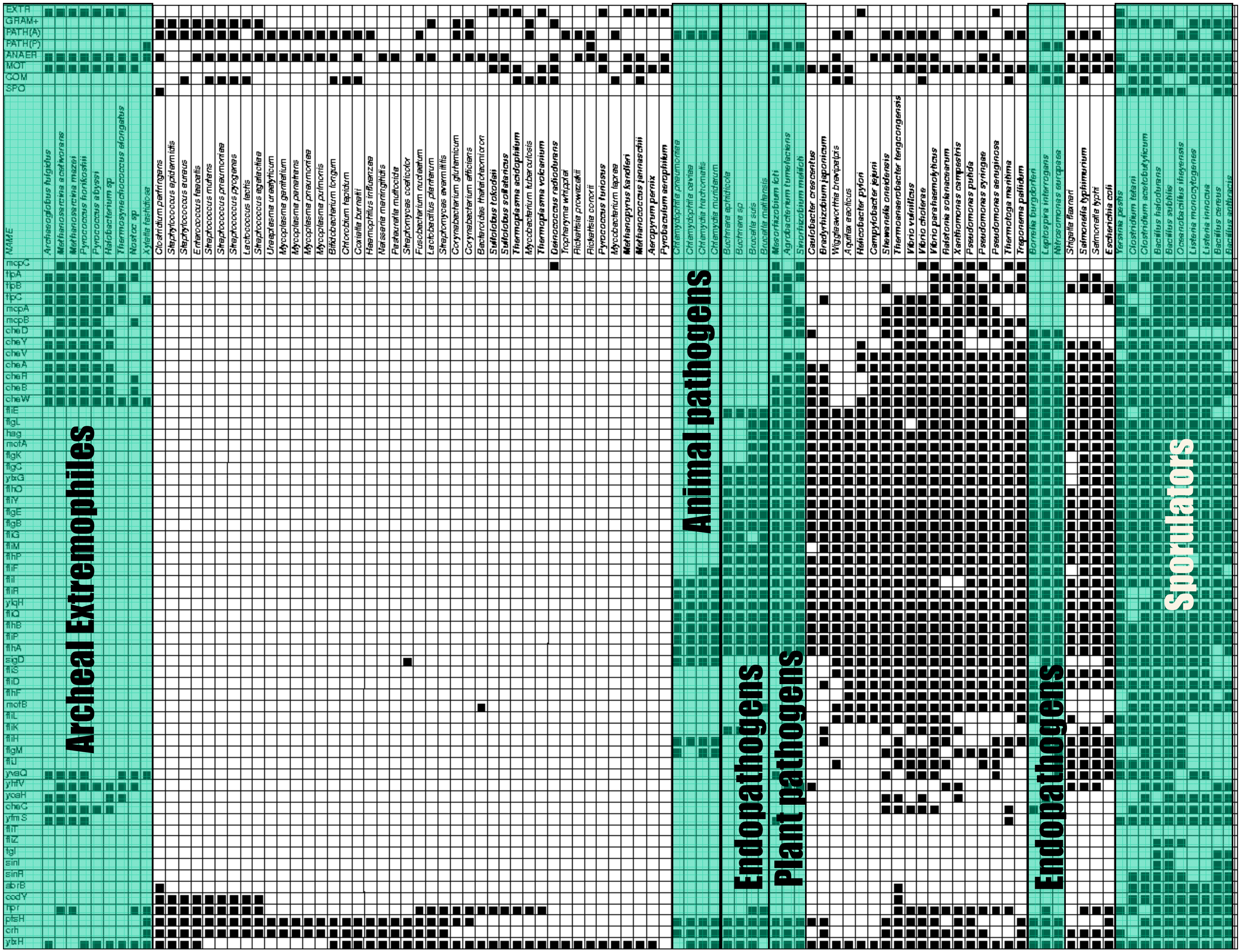
Organization of Phenotypes?



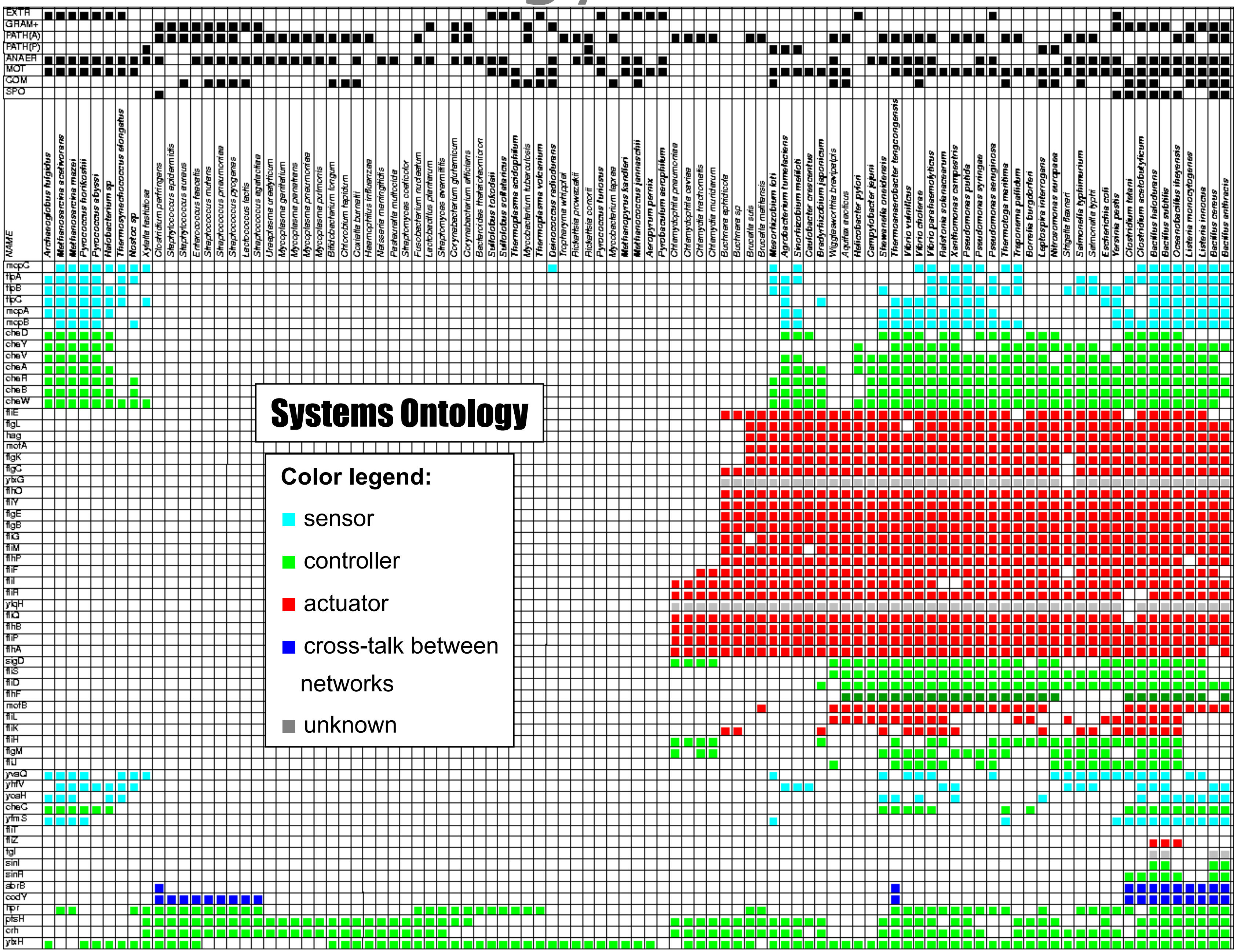
Clusters are functionally coherent



Different modules for different lives

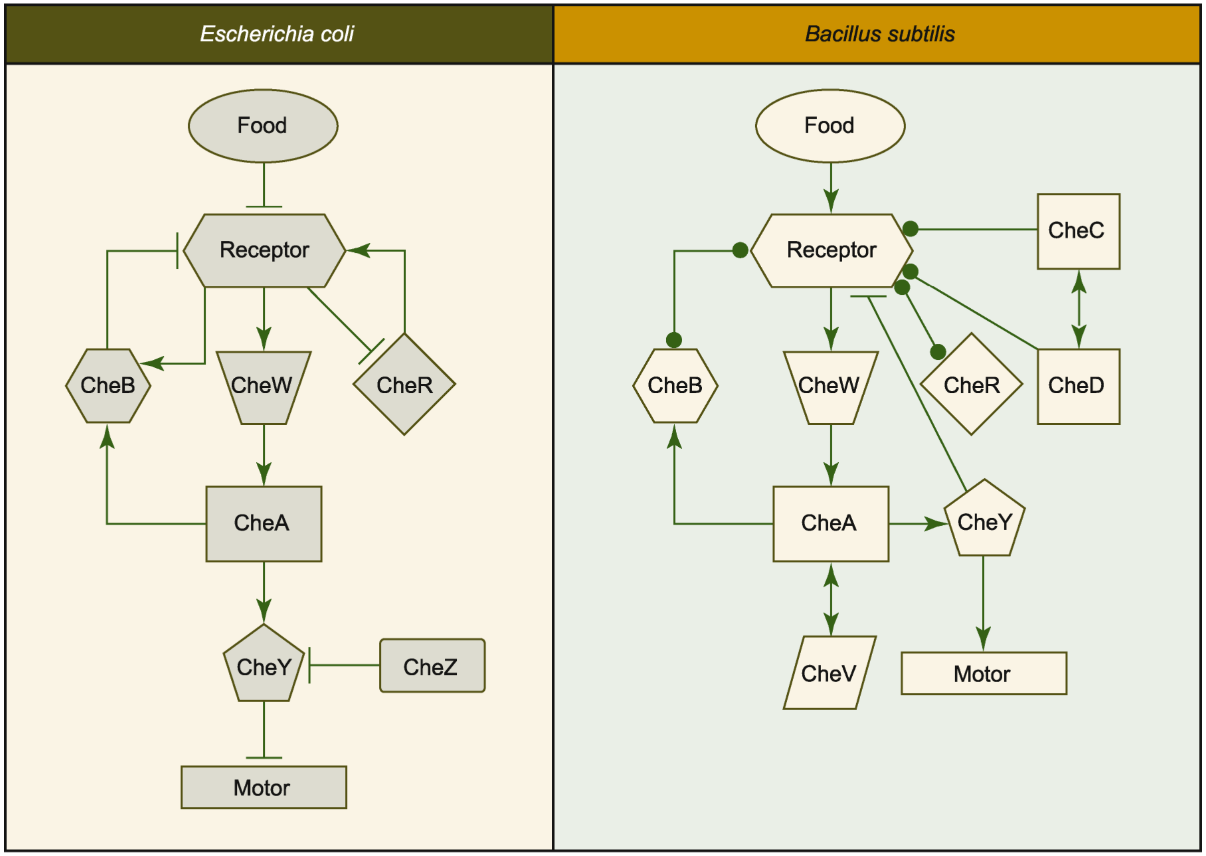


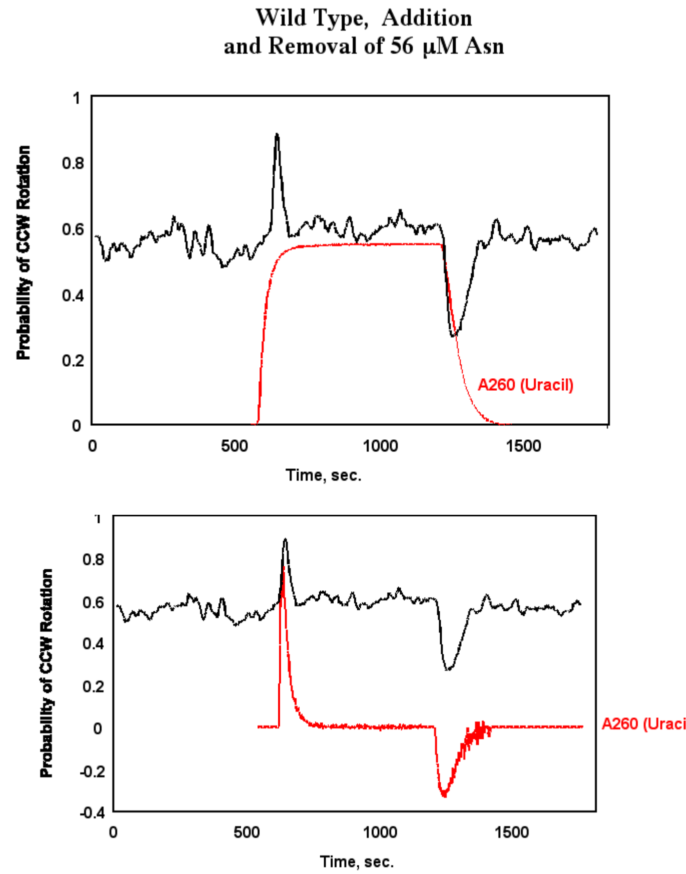
What Ontology Recovers Modules?



Comparative analysis is especially important

Rao, CV, Kirby, J, Arkin, A.P. (2004) PLOS Biology, 2(2), 239-252





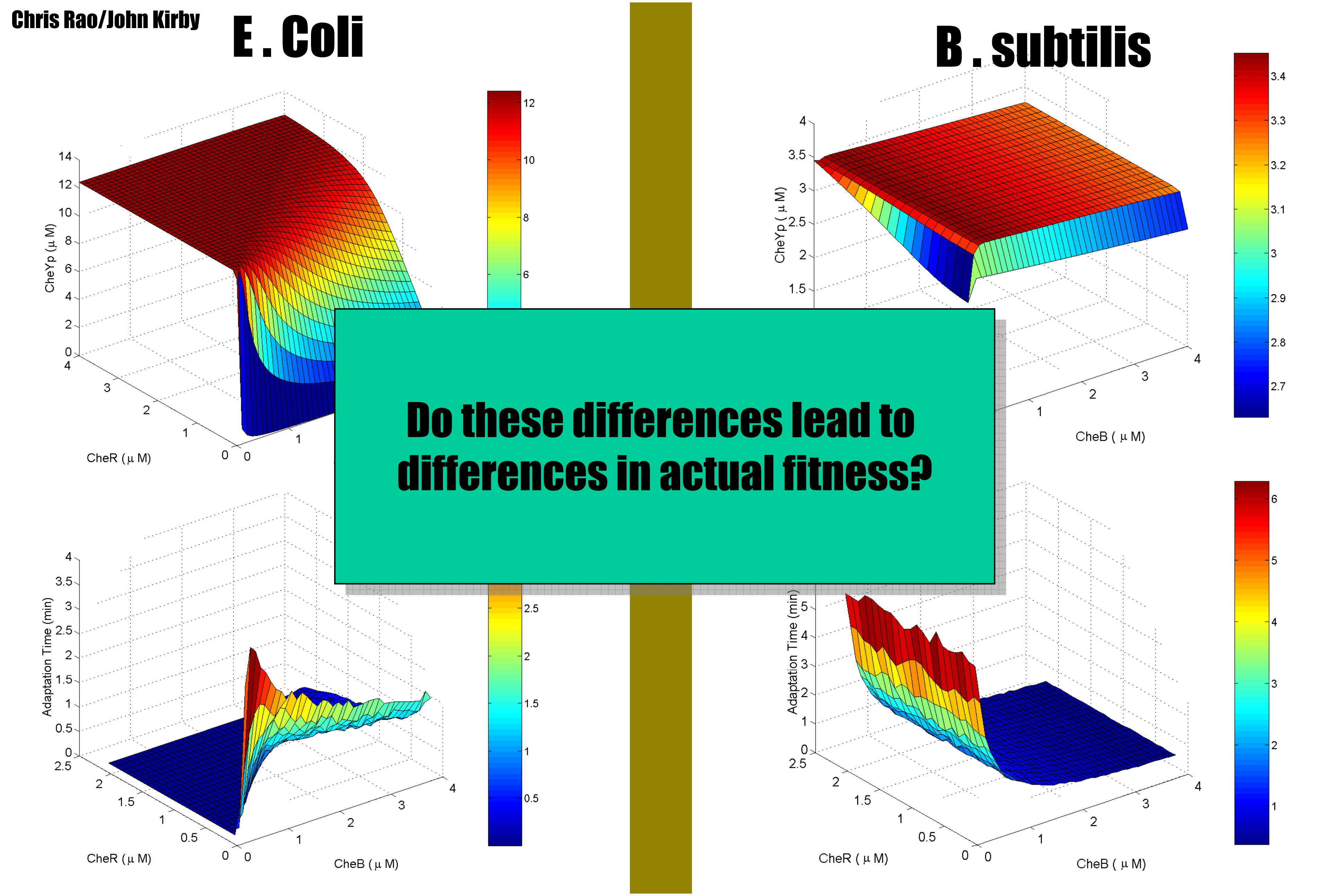
These are the homologous chemotaxis pathways in E.coli and B. subtilis

They have the same wild-type behavior.
Different biochemical mechanisms.
Different robustnesses!

Chris Rao/John Kirby

Differences in robustness

Chris Rao/John Kirby



Design of “Viruses”

Leor Weinberger, David Schaffer

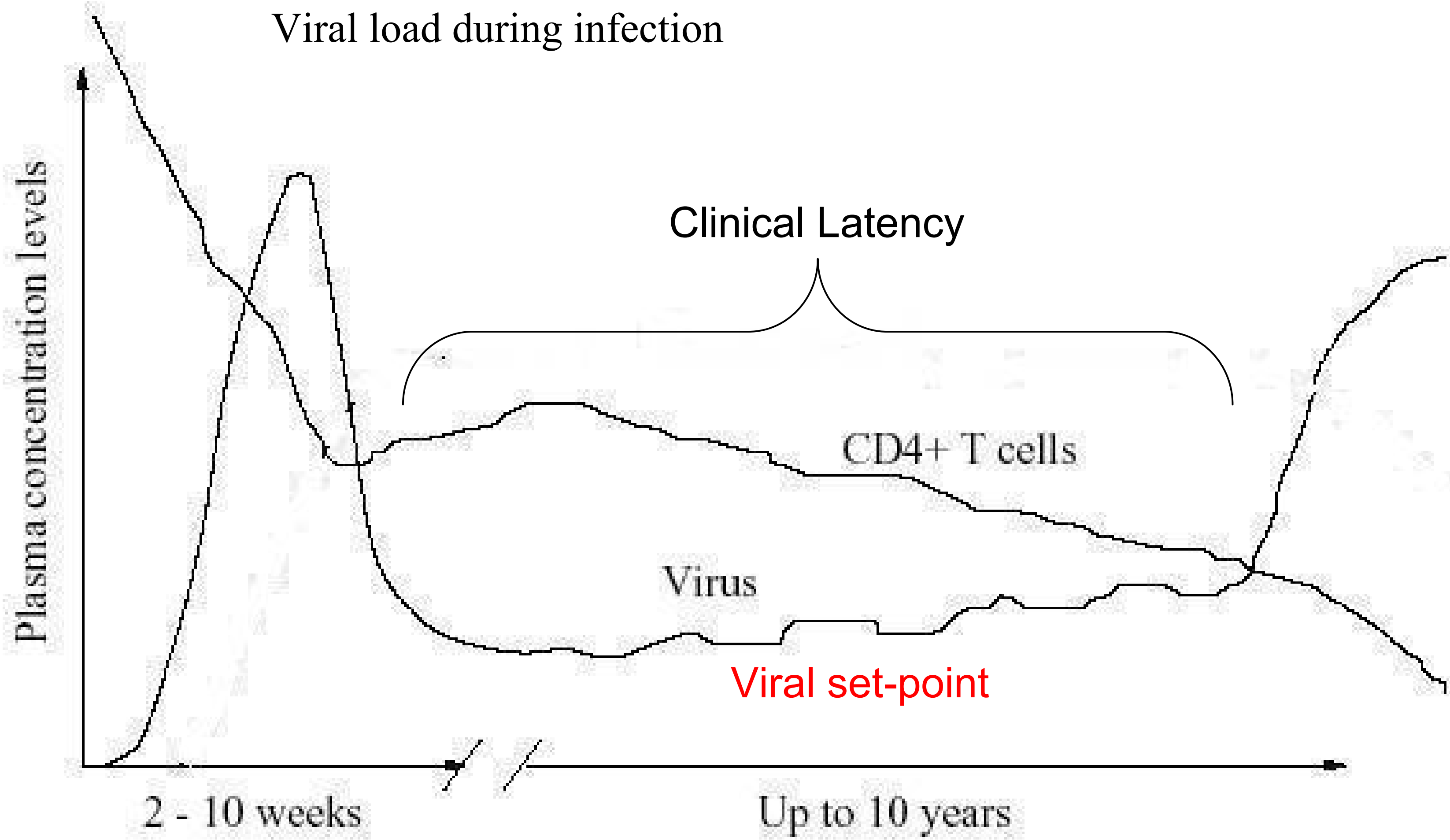
BACKGROUND

- What is AIDS?
  - Actual clinical syndrome when immune system breaks down
  - $CD4^+$ T cell count < 200 cells μL & Opp. Infections present
- How does HIV cause AIDS?
  - $CD4^+$ T cell killing and eventual exhaustion of T cell replenishment machinery (currently most accepted of 3 theors.)
- What is the treatment?
  - HAART (Highly Active Anti-Retroviral Therapy) a.k.a. triple-drug cocktails reduce viral production and increase CD4 count.
  - HAART decreases the rate of $CD4^+$ cell turnover.
  - HAART delays progression to AIDS.

PROBLEMS WITH HAART

- Serious side-effects
  - Many patients can't tolerate many of the 18 available drugs
  - Liver, Spleen, Pancreatic damage
  - Fat redistribution (humpback condition)
- Expensive
  - $15,000/year for drugs alone
  - Majority of 40 million infected live in 3rd world.
- Viral resistance
  - arises on average after 18 months of therapy
- Latent reservoirs of infection
  - resting cells not actively producing virus and thus not affected by HAART
  - Estimates ⇒ more than 50-60 yrs. of continuous HAART to purge latent reservoirs and eradicate HIV from patient.
  - Reactivation with IL-2, IL-7 did not work, prostratin is something to hope for– but other things have worked in mice before.
  - Vaccines and other treatments not offering much hope

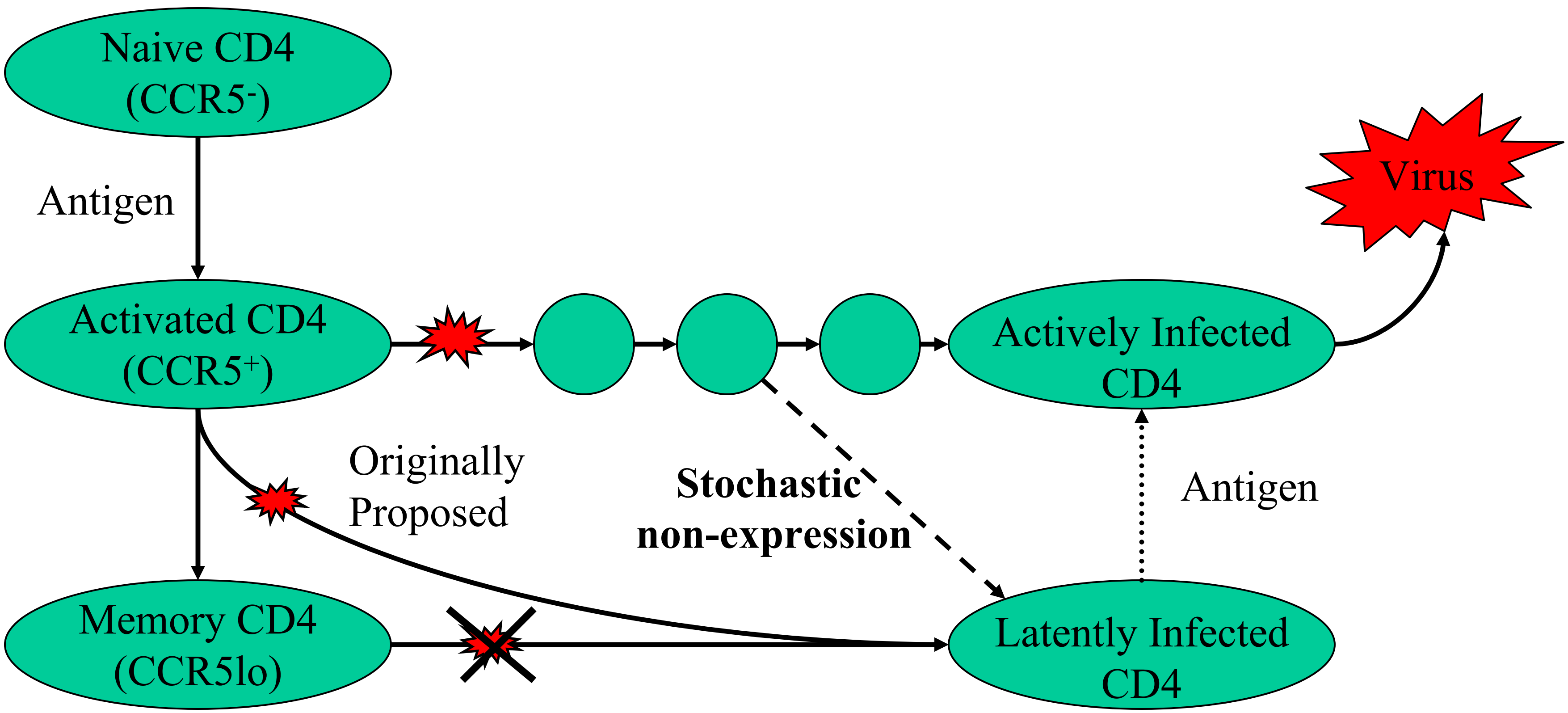
HIV-1 Disease Dynamics



Perelson & Nelson, SIAM Review, **41**:3-44 (2000)

HIV-1 Post-integration (proviral) Latency

- Identified reservoir is Memory $CD4^+$ T cells having integrated proviral DNA
- Population is small (few hundred cells/individual) and long-lived (lower limit estimates ⇒ 80 yrs. of continuous HAART to purge latent reservoirs and eradicate HIV from patient.
- Reactivation (for e.g. with IL-2) does not work



Designing a therapy:Specifications

- Exploit HIV tendency to go latent
- Easy to administer
- Easy compliance
- Easy to deliver (perhaps too easy)
- Persistent
- Surveillant: waits for HIV to begin to express.

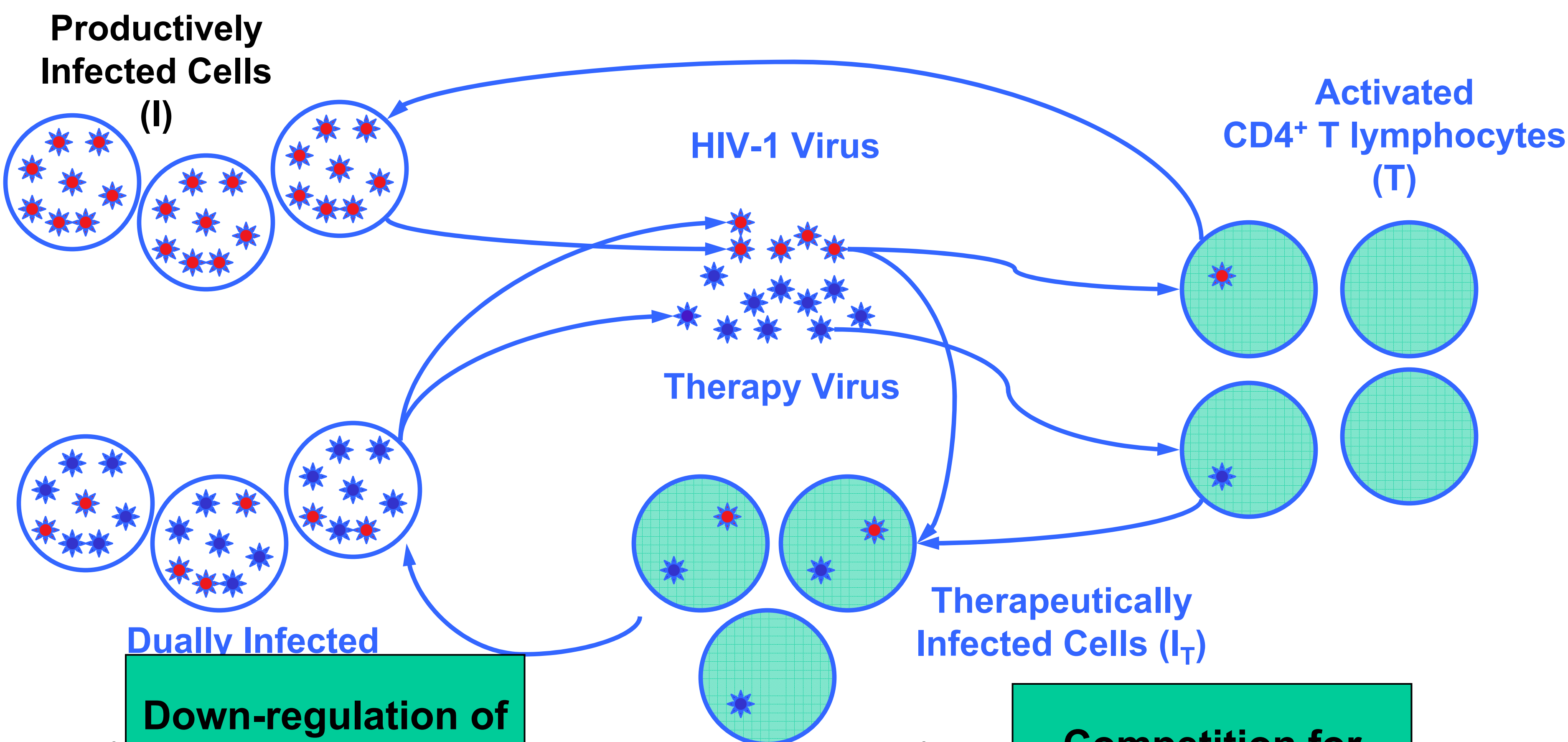
What’s the idea?

- Use a parasitic conditionally-replicating lentivirus
- Gut HIV-1 to create a harmless lentivirus
  - Lentiviruses seem not to be too bad in causing problems simply due to integration.
  - Cells that have lentiviruses can express almost unlimited virions if the cytotoxic viral proteins are removed
- Remove all capsid proteins to prevent our virus from being able to propagate by itself
- Add agents that allow it to both down-regulate HIV, yet steal the phage-coat made by HIV so our therapy can propagate.

How could we do it?

- **Use the native Ψ packaging element to compete efficiently with HIV for it's coat**
- **Use the woodchuck element, for example, to increase our viral RNA stability relative to HIV**
- **Express molecules to down-regulate HIV expression**
  - **Tat decoys**
  - **Competitors for Tar-binding**
  - **Targets for host-proteins necessary for HIV life-cycle**
  - **Combinations of these**
  - **(Currently implemented by siRNAs and a few other tricks)**
- **But will this work?**
  - **How much do we have to compete with HIV to steal the majority of phage coat**
  - **How much should we down regulate HIV for therapeutic effect**

Expanded Clinical Model with crHIV-1 vector included



Down-regulation of HIV-1 by crHIV-1

Competition for Virion production

$$\dot{T} \; \ldots \; - k V_T T$$

$$\dot{I} = k V T - \delta I$$

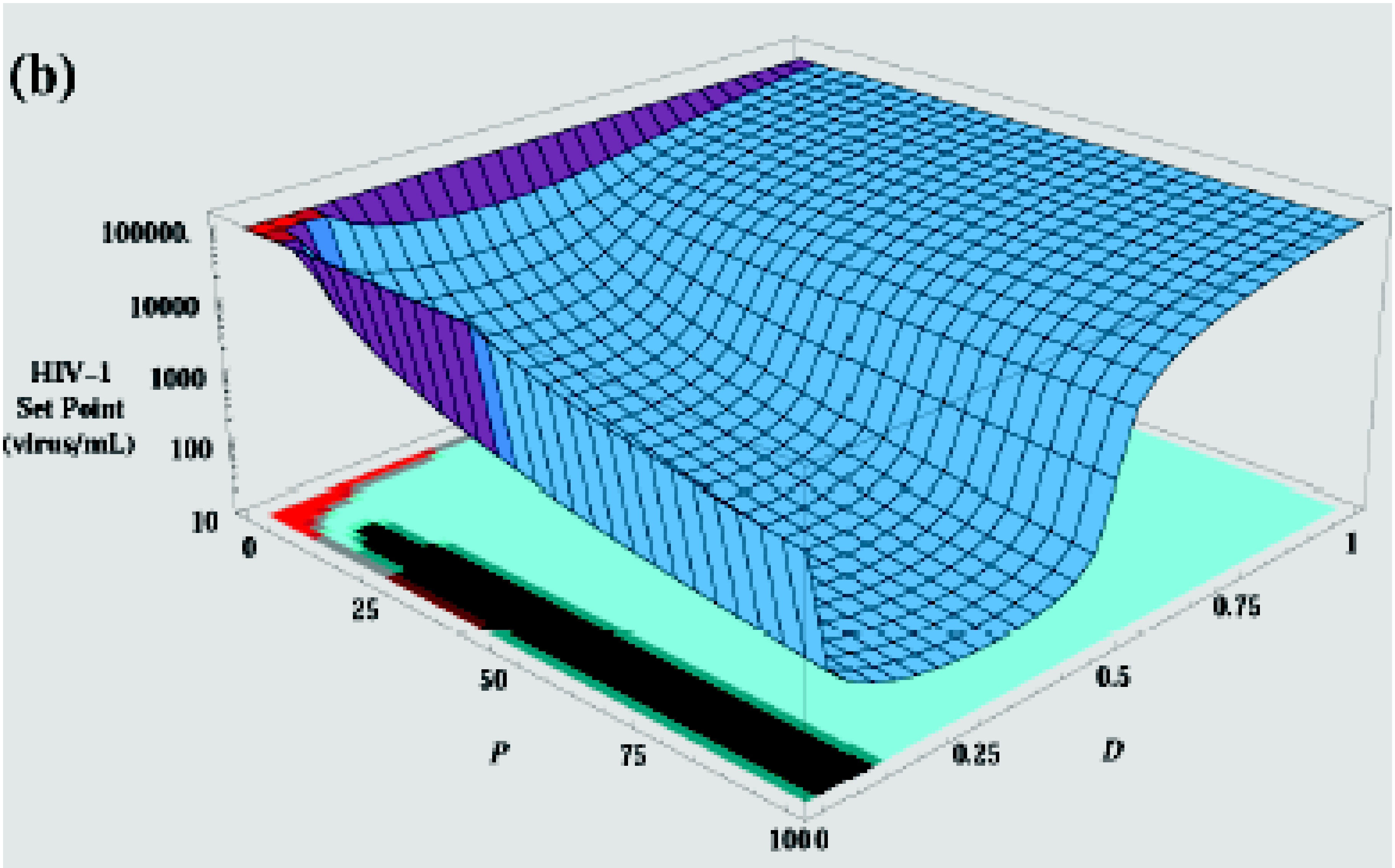
$$\dot{V} = n \delta I - c V + D n \delta ' I_D$$

$$\dot{I}_T = \; \ldots \; V I_T$$

$$\dot{I}_D = k V I_T - \delta ' I_D$$

$$\dot{V}_T = P^2 D n \delta ' I_D - c V_T$$

Steady state HIV-1 levels after crHIV-1 administration



Discussion

1. **The model gives a quantitative analysis of diverse gene therapy approaches; the analysis is independent of the specific of anti-viral (down-regulating) gene used.**

2. **Use of multi-point and host-factor repression decreases mutational escape dramatically.**

3. **There does exist a parameter regime where a crHIV-1 therapeutic vector can persist along with HIV-1. A simple function R_0^T can be used to design crHIV-1 vectors that persist in vivo.**

4. **crHIV-1 can achieve a 3 log decrease in HIV-1 set point when packaging efficiency is 20 fold greater than HIV-1.**

5. **Not necessarily "better" to have a very efficient inhibitor of HIV-1 production.**

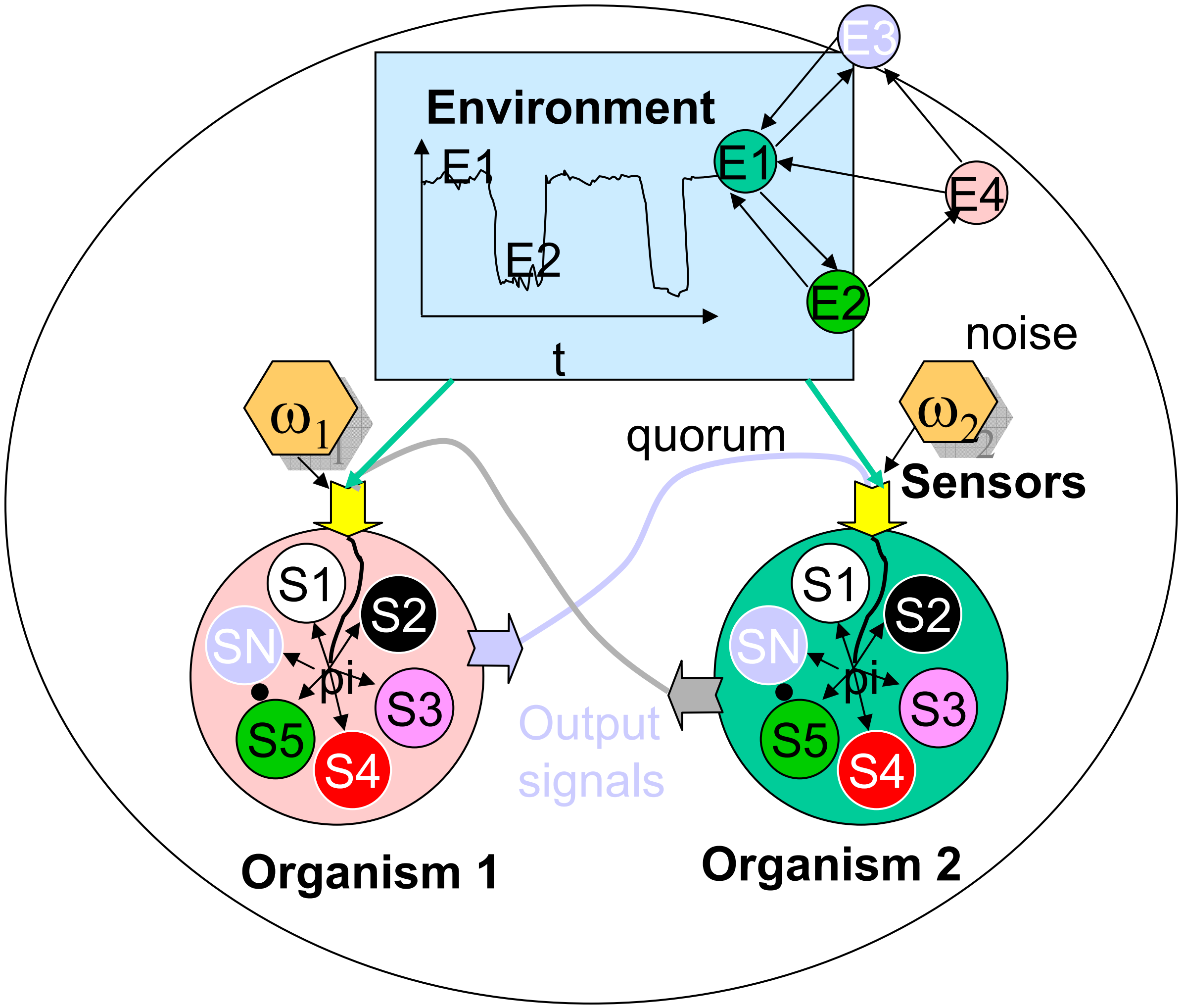
Biological issues

- Introduction of a new lentivirus into a human host might cause pleiotropic disease (lymphoma,e.g.) (Unlikely)
- Uncontrolled mutation of HIV and therapy virus still leads to resistant virus (though less so)
- It is a stochastic non-linear control problem– we have little theory to help us.
- Introduction of new virus changes the viral ecosystem

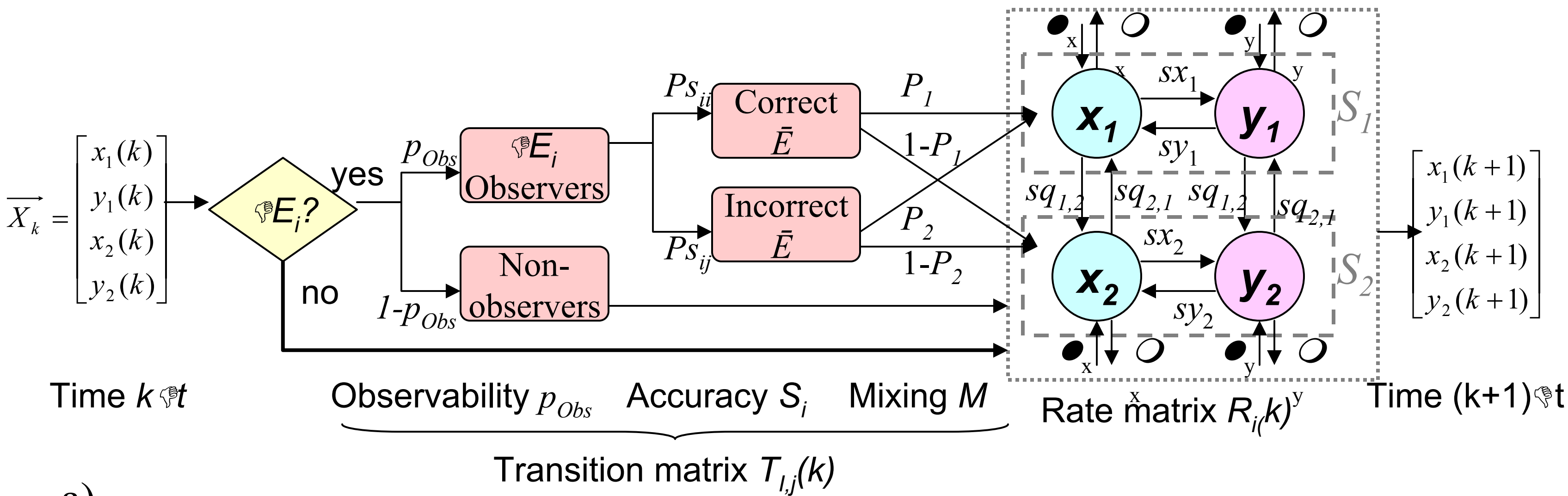
Social Issues

- Theoretically, our therapy virus is sexually transmittable
- Epidemiological studies show that when medical treatments are introduced, promiscuity increases dramatically in many cases.
- The barrier to production of virus is relatively low
- Who will support or profit from a self-propagating persistent therapy?
- Is there anyway of assessing cost-benefit or risk when using engineered organisms such as this one?

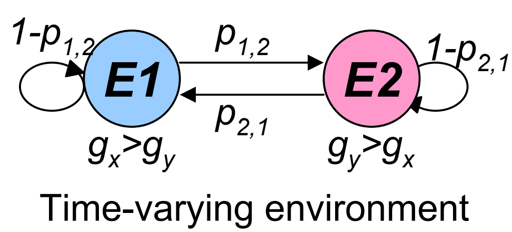
The game of life



Formal Model

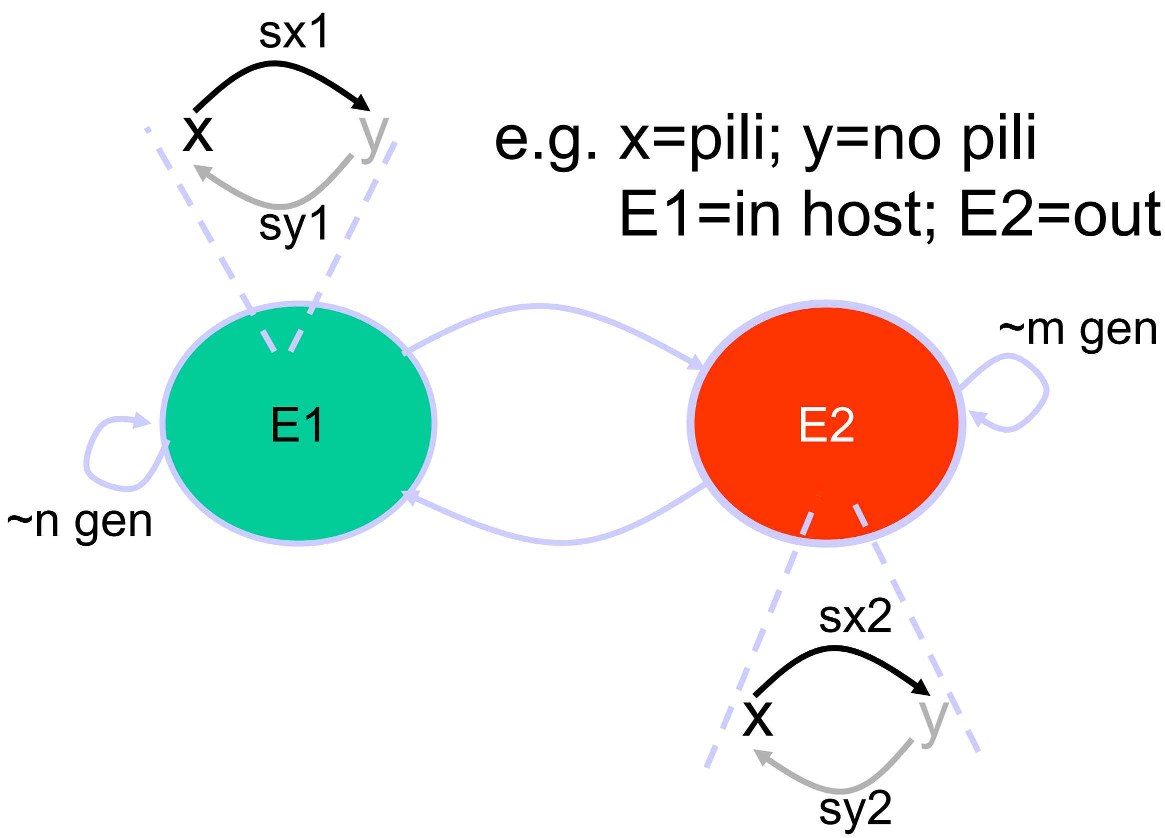


a)



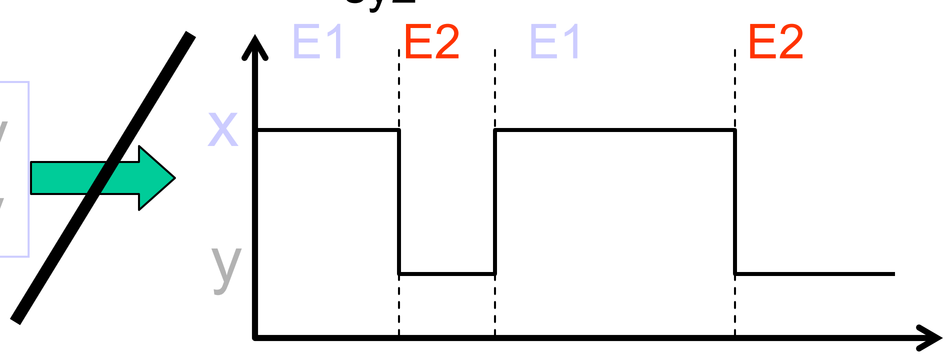
b)

Example: two environments, two moves, no sensor



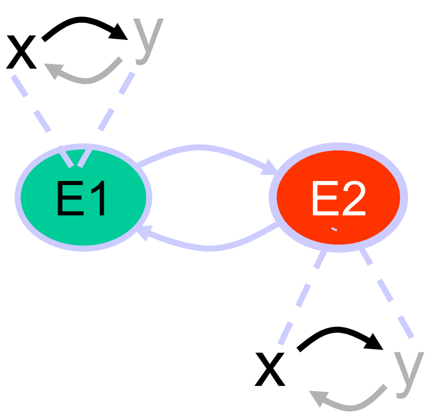
e.g. x=pili; y=no pili
E1=in host; E2=out

IF E1: selects for x, against y
E2: selects against x, for y



With no sensor, the options are…

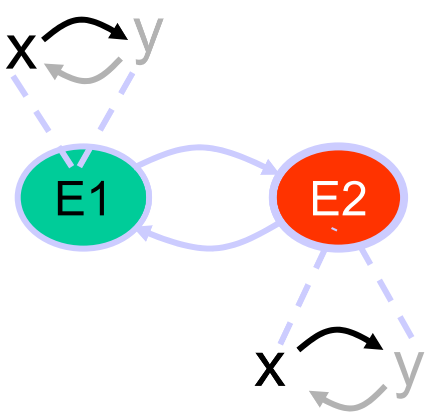
Denise Wolf, Vijay Vazirani

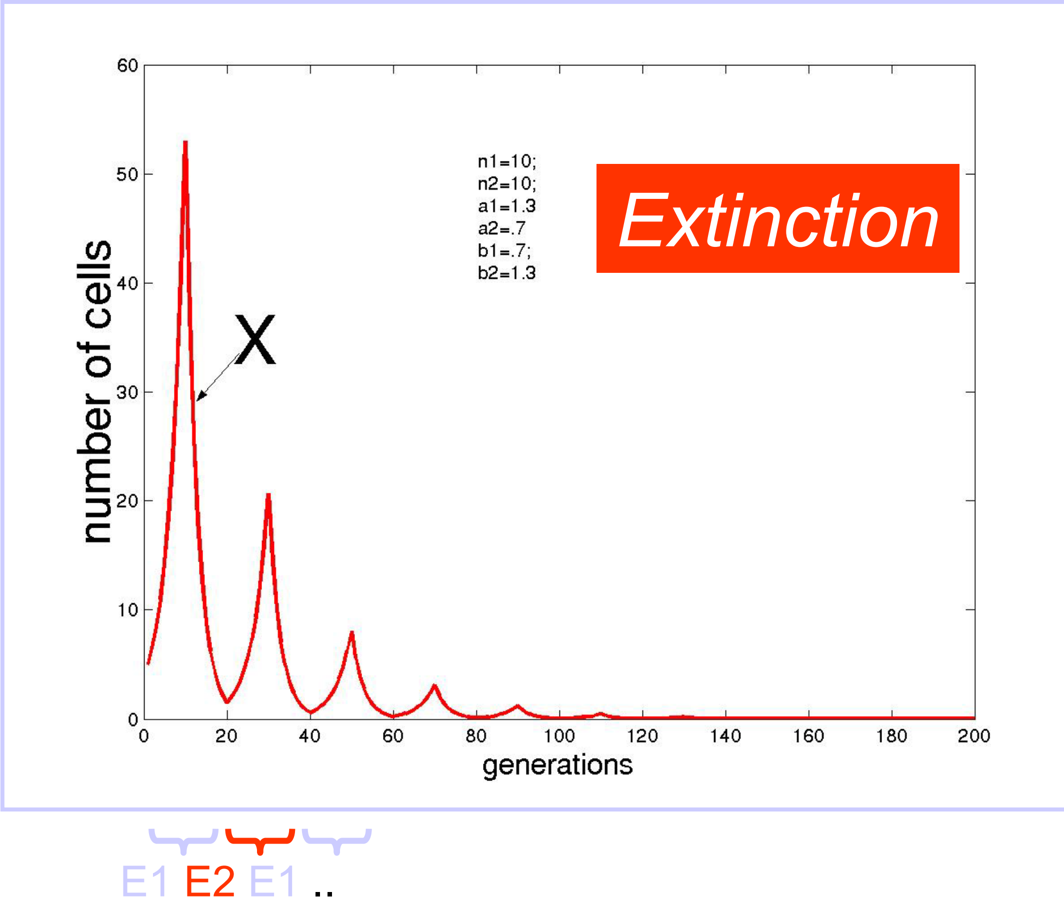


1. ALL cells in state x
2. ALL cells in state y
3. Statically mixed population (some x, some y)
4. Phase variation of individual cells between x and y

With no sensor, the options are…

Denise Wolf, Vijay Vazirani





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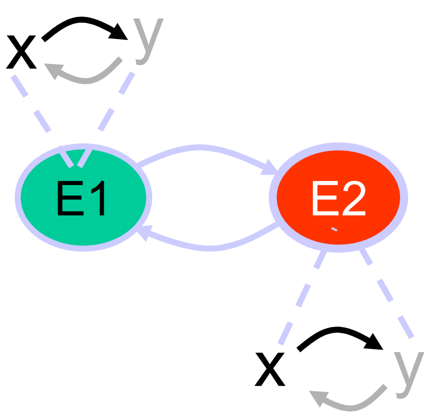

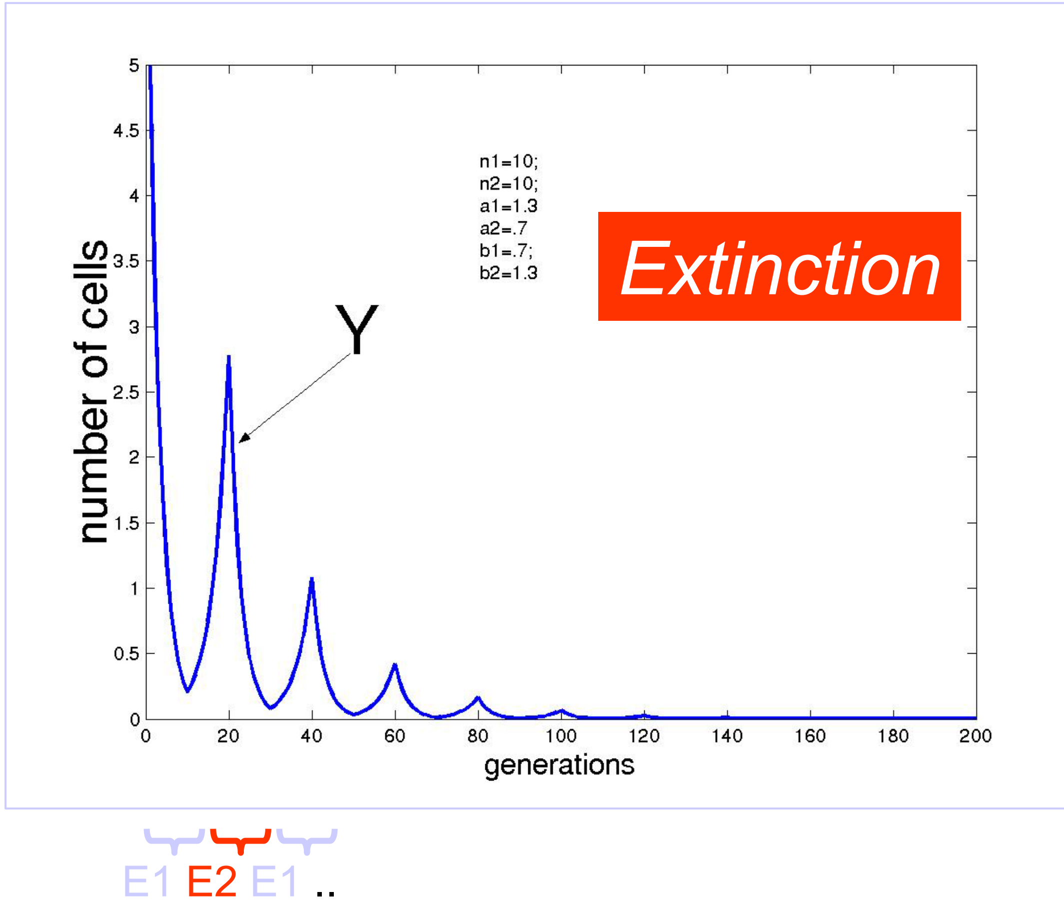

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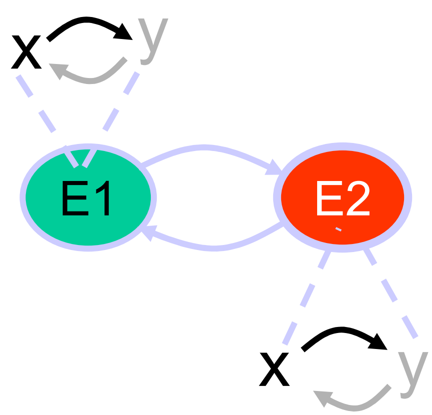

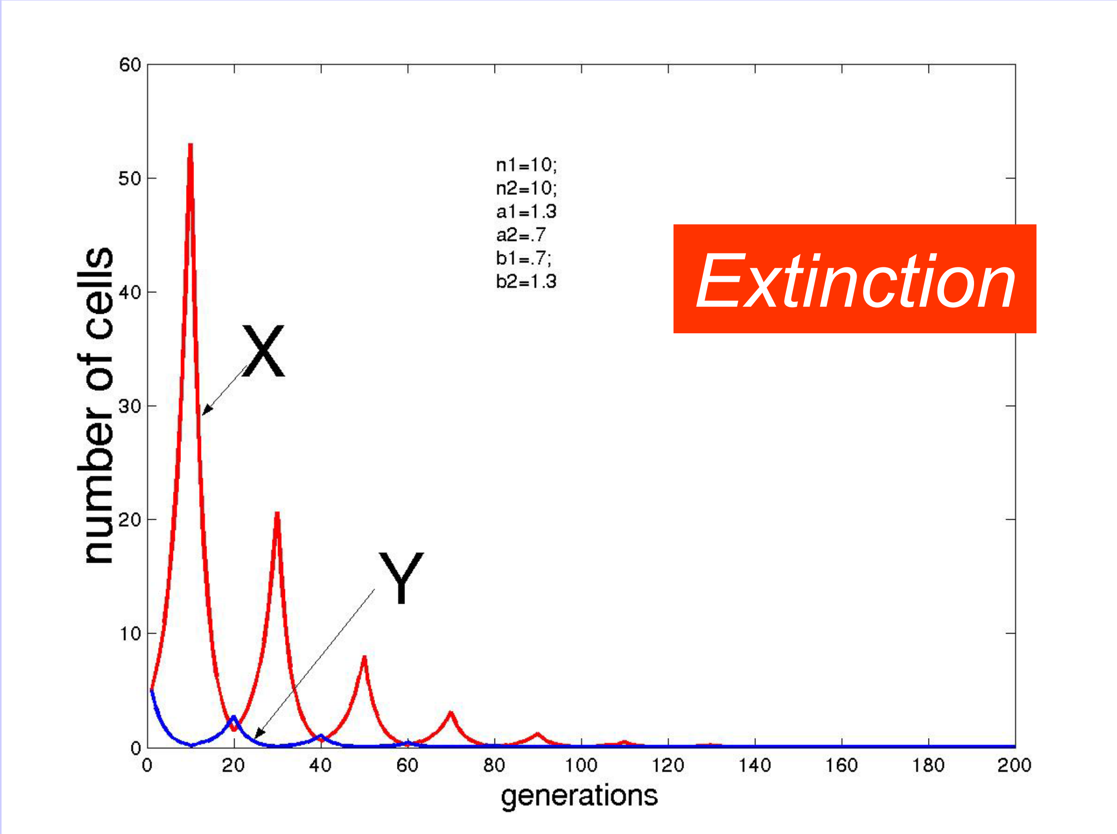

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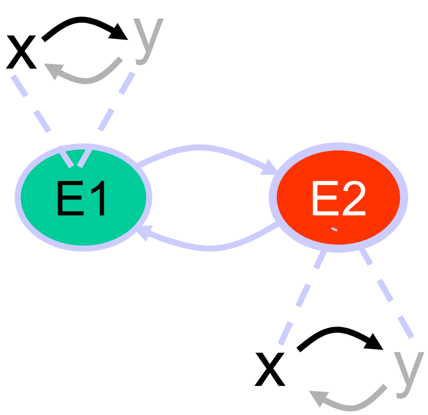

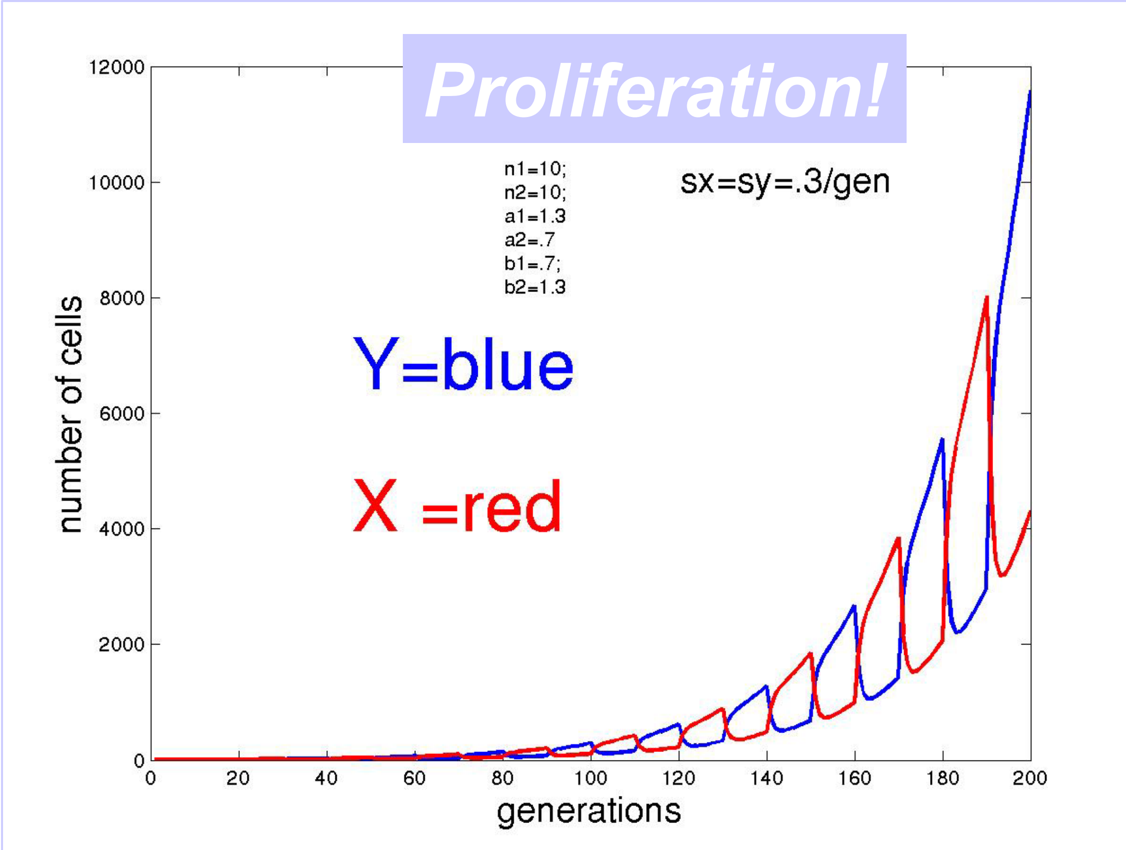

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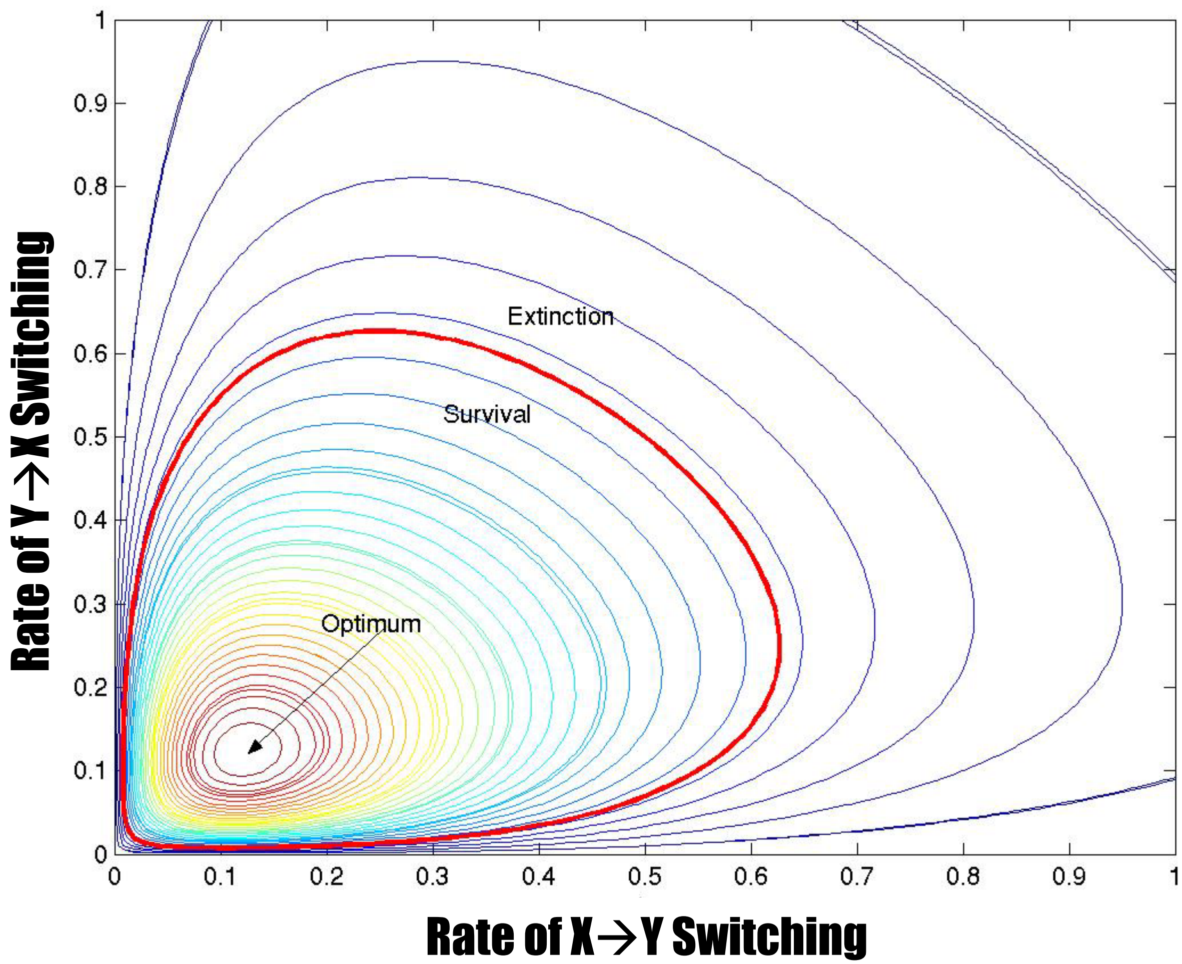
Phase variation for survival



This is a Devil's compromise: **Phase-variation behavior is not optimal** in any one environment but necessary for survival with noisy sensors in a **fluctuating** environment.

Denise Wolf, Vijay Vazirani

Learning Environment from Cell State

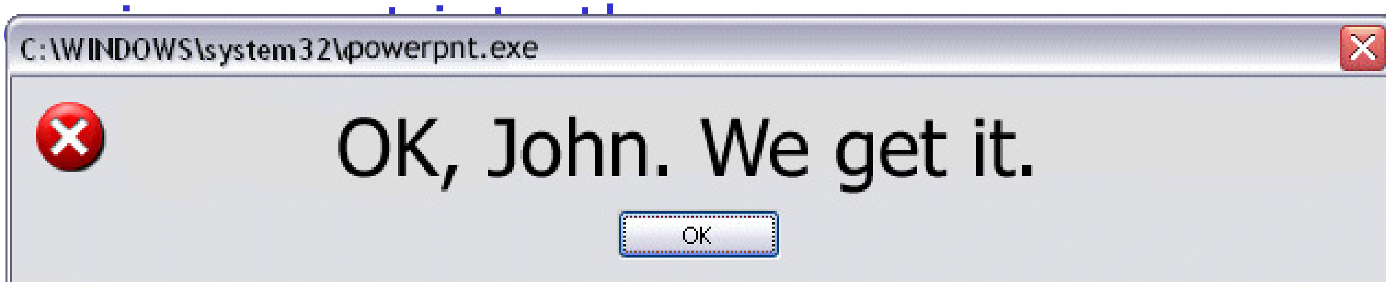
Diversity Strategies

Strategy	Sensor profile	Environmental profile
Random Phase Variation (RPV)	No sensors	•Devil's Compromise (DC) lifecycle: time varying environment with different environmental states selecting for different cell states. •Optimal switching rates a function of lifecycle asymmetries and environmental autocorrelation. •Time variation required (spatial variation insufficient).
	O=Low prob. observable transitions over DC or extinction set.	
	D=Long delays relative to env. transition times.	
	Perfect sensors	Frequency dependent growth curves with mixed ESS.
Sensor Based Mixed	O=High prob. observable transitions; A=Poor accuracy	•Devil's Compromise lifecycle. •Asymmetric lifecycle required. •Optimal mixing probabilities biased toward selected cell-states in dominant environmental states.
Sensor Based Mixed; LPF	O=High prob. observable transitions; A=Poor accuracy. N=High additive noise.	
Sensor Based Pure	O=High prob. observable transitions; A=High accuracy; or moderate accuracy and low noise N.	Temporally or spatially varying environment with each environmental state selecting for a single cell state.
Sensor Based Pure; LPF	O=High prob. observable transitions; A=Moderate accuracy. N=High additive noise.	

Robustness and Fragility

- The stratagems of a cell evolve in a given environment for robust survival.

- Evolution writes an internal model of the



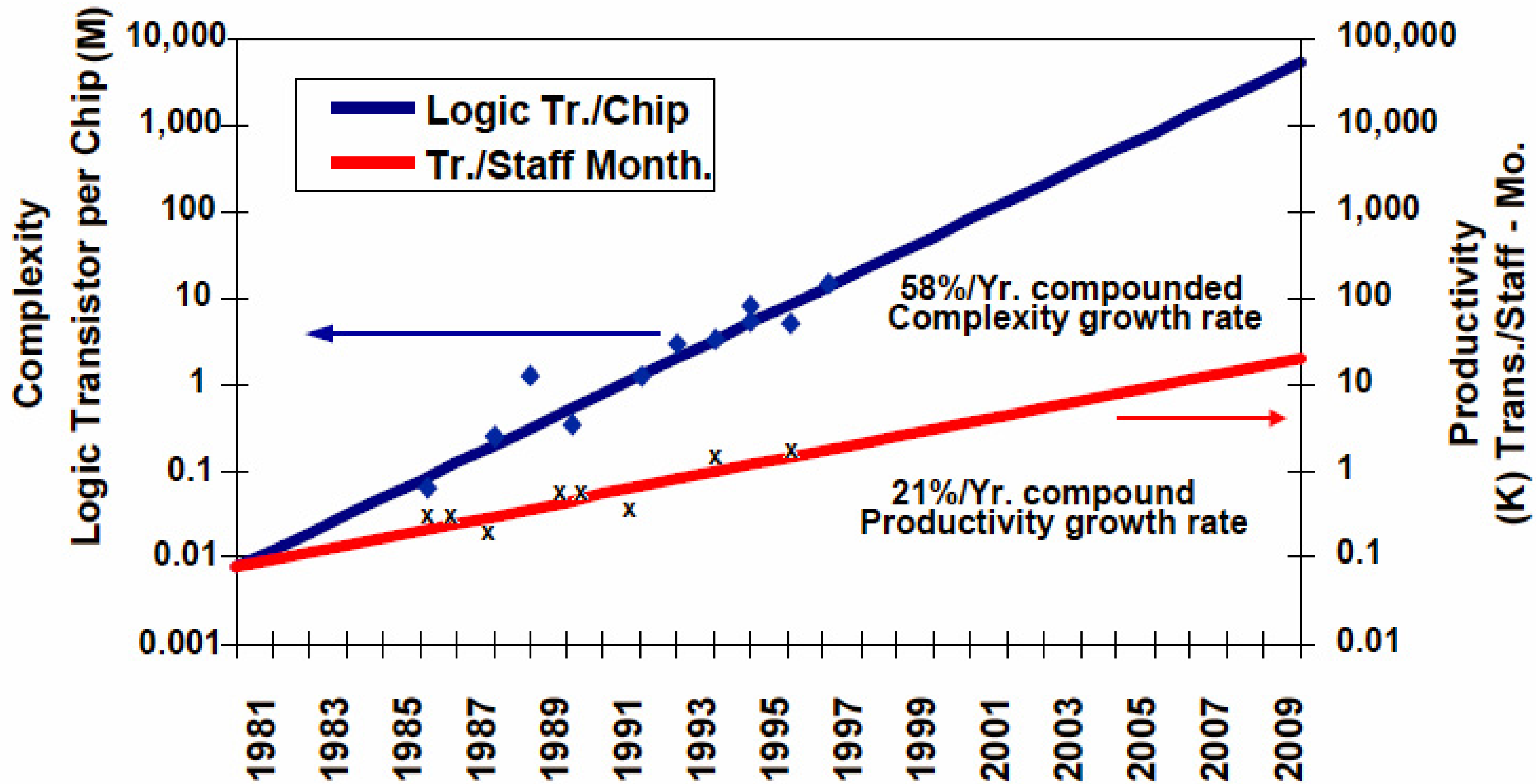
-
  - ...here are evolvable designs)
  - And certain random changes in its process structure.

- It's not clear if cells are more or less robust than, say, this laptop.

Engineering beyond the bioreactor

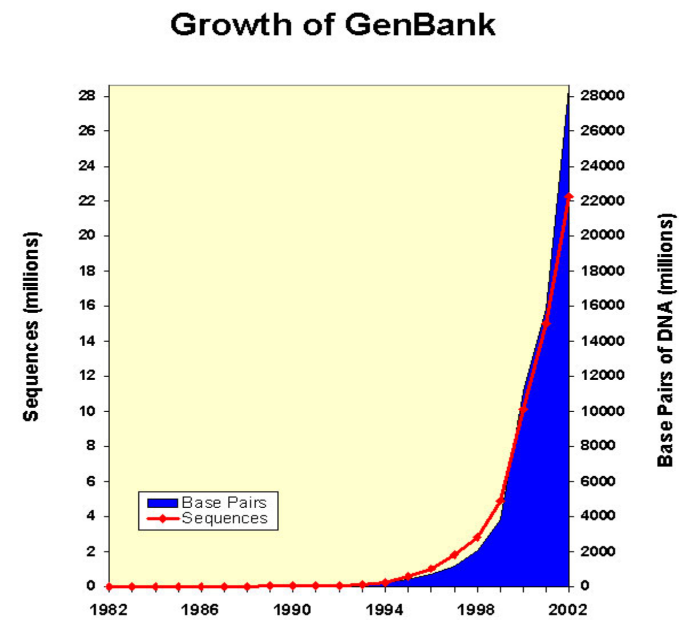
- Engineering organisms for use outside the controlled production of biochemicals or to instrument them with a few fluorescent proteins requires a better understanding of the cellular system and its evolution.
  - Engineering microbes for drug production
  - Engineering microbes for bioremediation
  - Engineering microbes for killing tumors
  - Engineering viruses for gene therapy
  - Engineering mosquitoes for malarial control
  - Engineering corn for agriculture
- Can we turn use a similar approach to engineering biological circuits as we do to engineering electronic circuits?

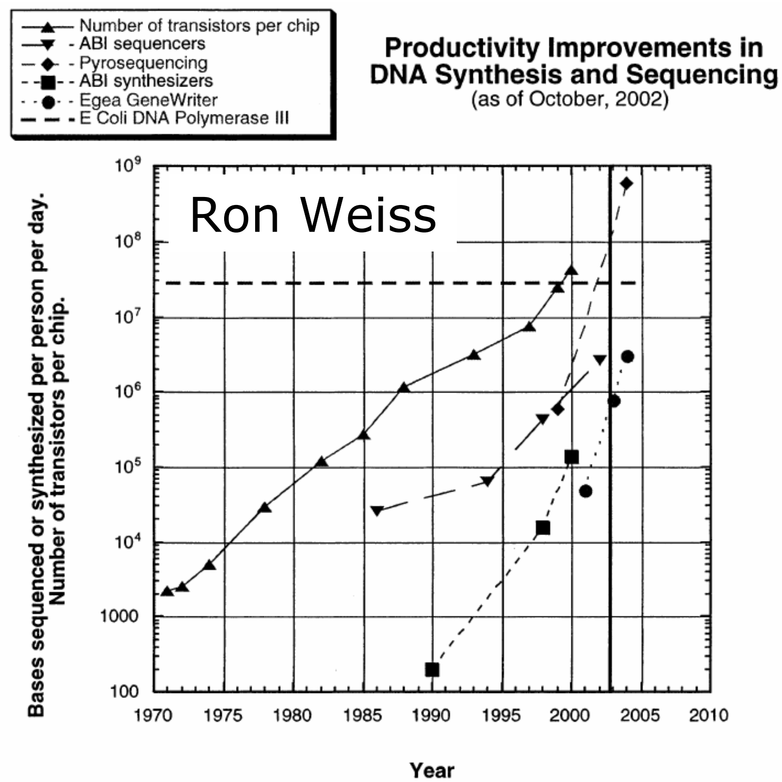
Productivity Trends



Exponentially More, Exponentially Cheaper Data

- Already large amounts of data, but incomplete
- Exponential increase: faster than Moore's law
- Key is translation of data into knowledge with tools that enable human understanding and help direct experimentation
- And GOOD theory of design and synthesis of biological networks!





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Happy Birthday, John!