Degrade-and-fire oscillations in synthetic gene networks

Lev Tsimring

BioCircuits Institute,

University of California, San Diego

UCSD

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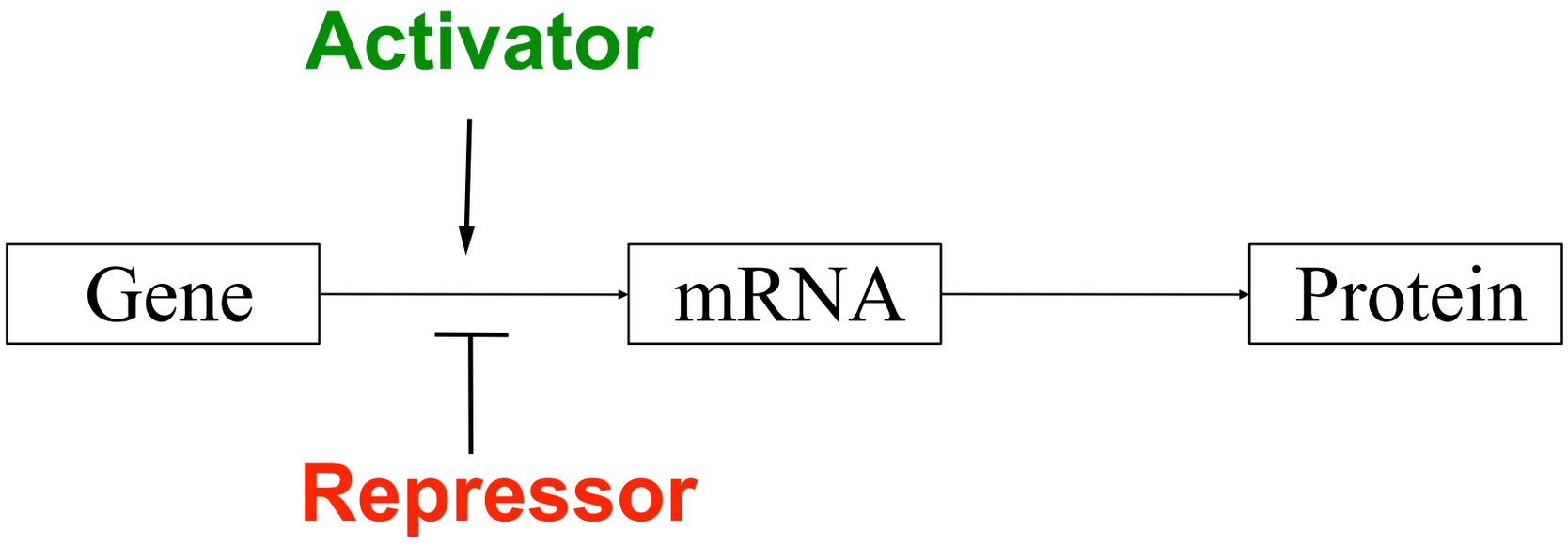
BCI

Central dogma



All cells have the same genes…
…So why cell differ from each other?

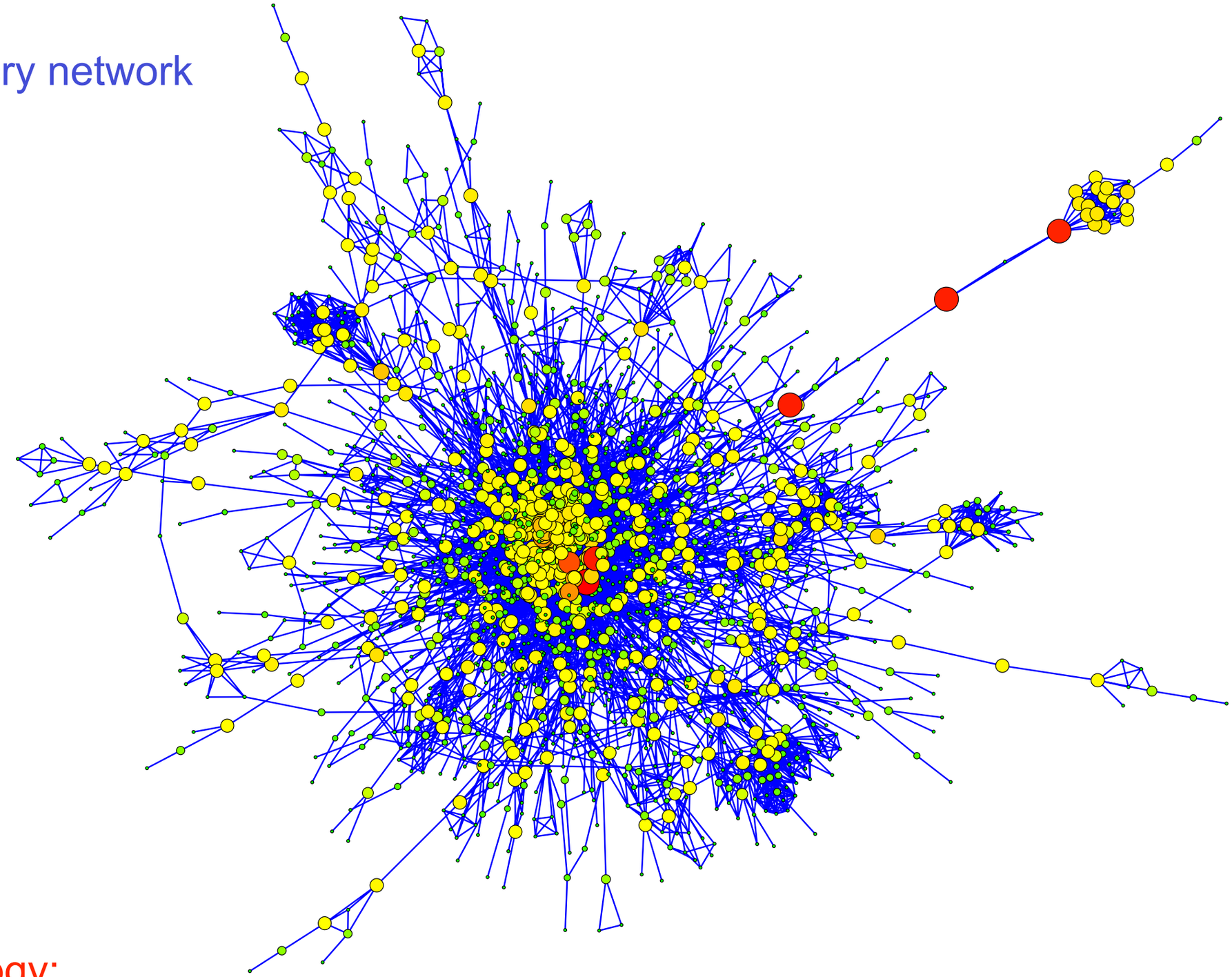
Transcriptional Regulation

Activator – increases rate of production

Repressor – decreases rate of production

Gene regulation circuits

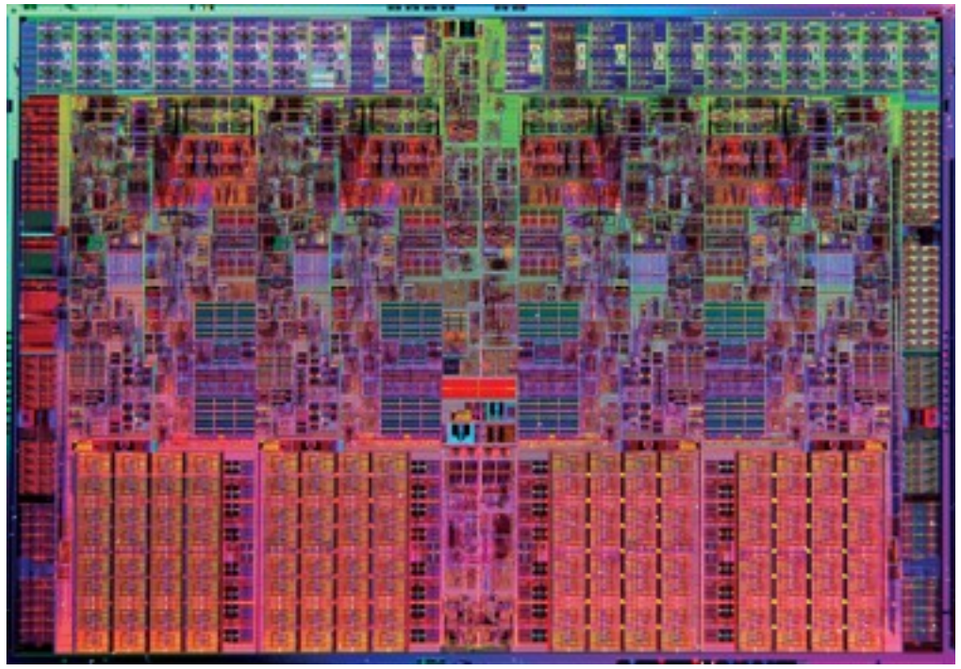
E.Coli regulatory network

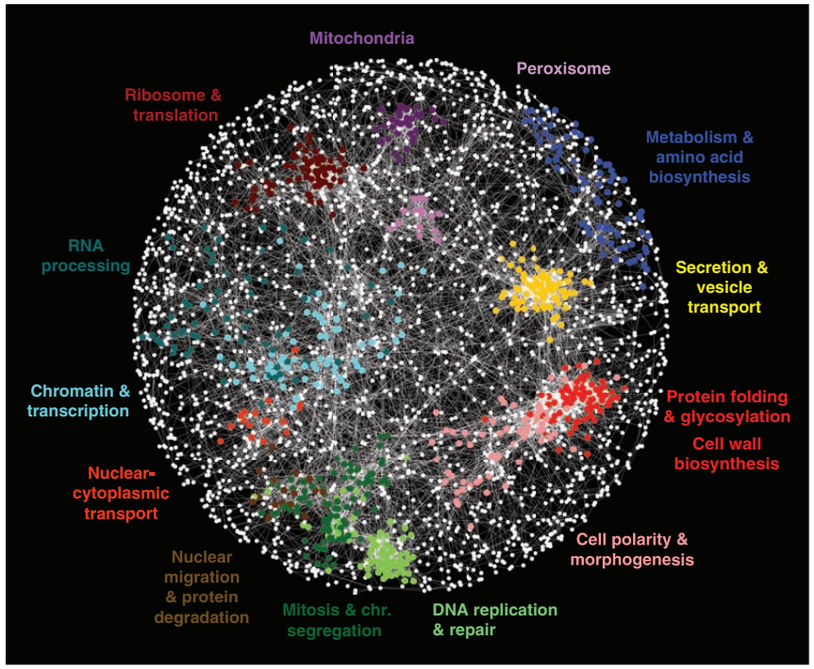


Systems biology:

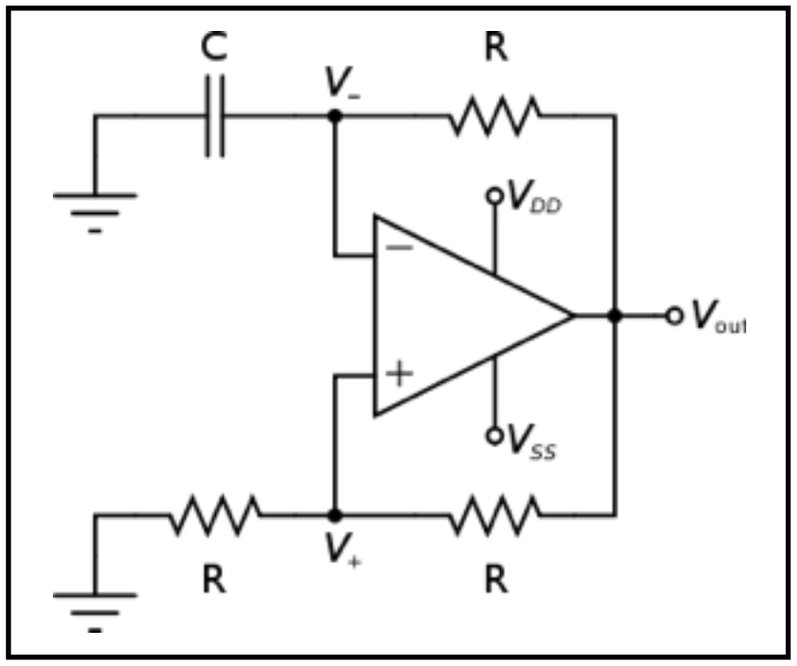
- Figure out all the genes (genomics)
- Figure out which genes and proteins interact (network reconstruction)
- Figure out the dynamics given the network

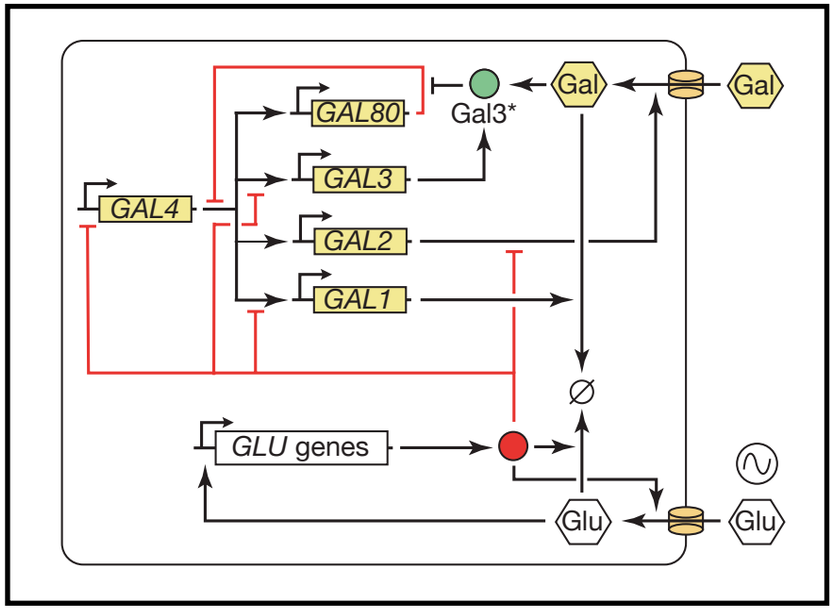
Systems Biology

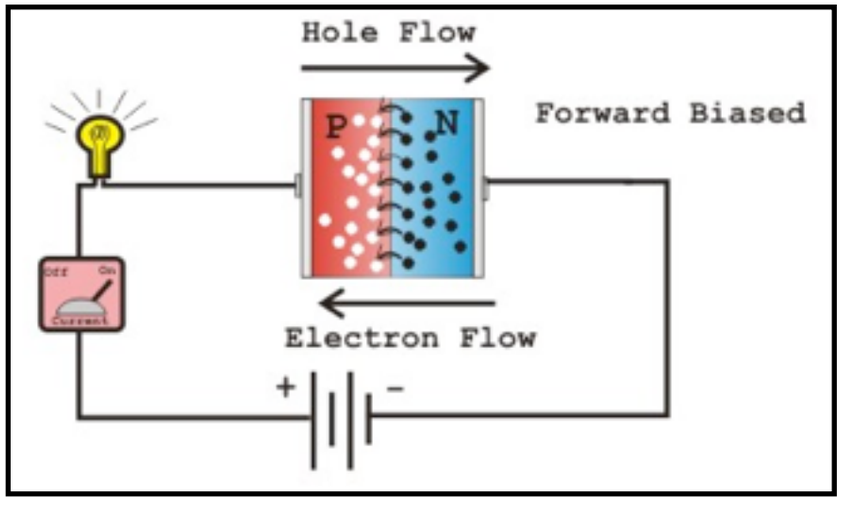




scale

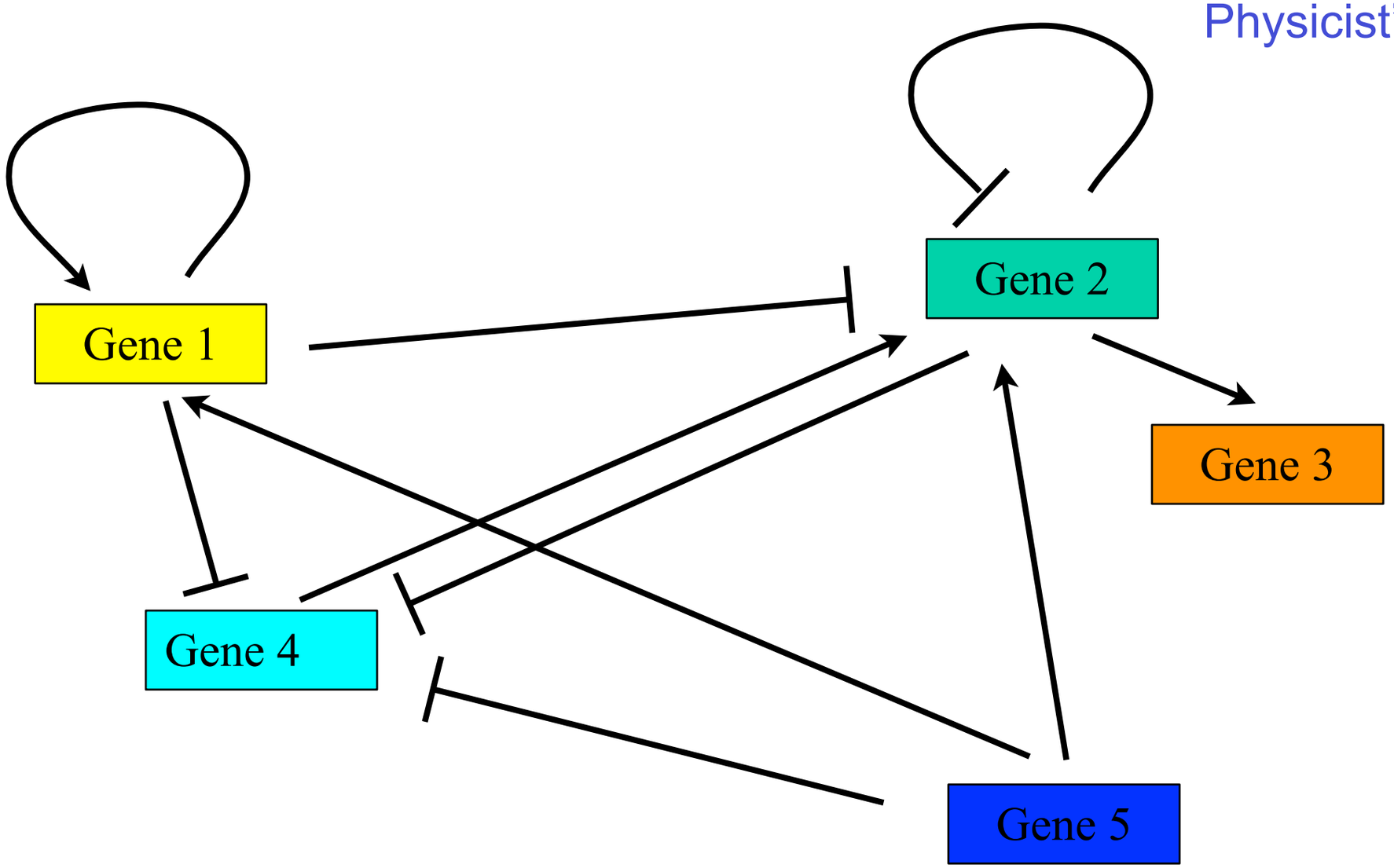






Gene regulation circuits

Physicist's cartoon



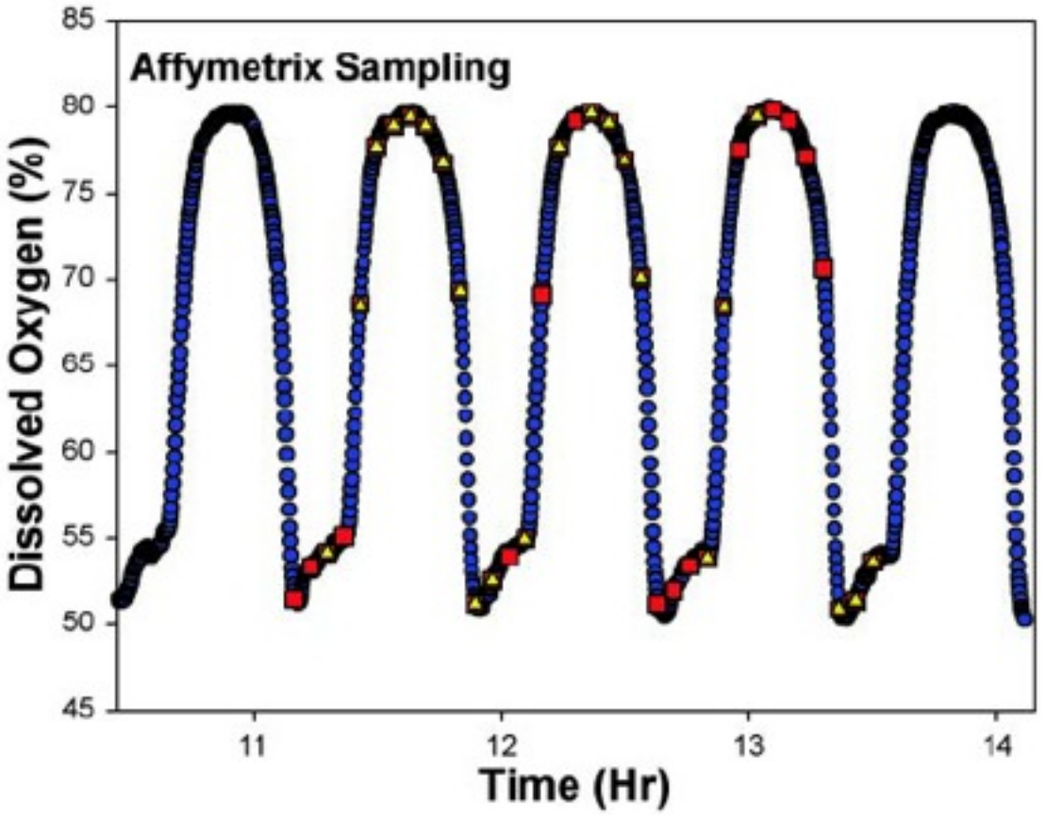
Dynamical and stochastic effects in gene expression

- Genetic circuits are never at a fixed point:
  - External signaling
  - Intrinsic noise
  - Extrinsic noise
  - Cell cycle; volume growth; division
  - Oscillations: circadian rhythms; ultradian rhythms, etc.

Ultradian clock in yeast

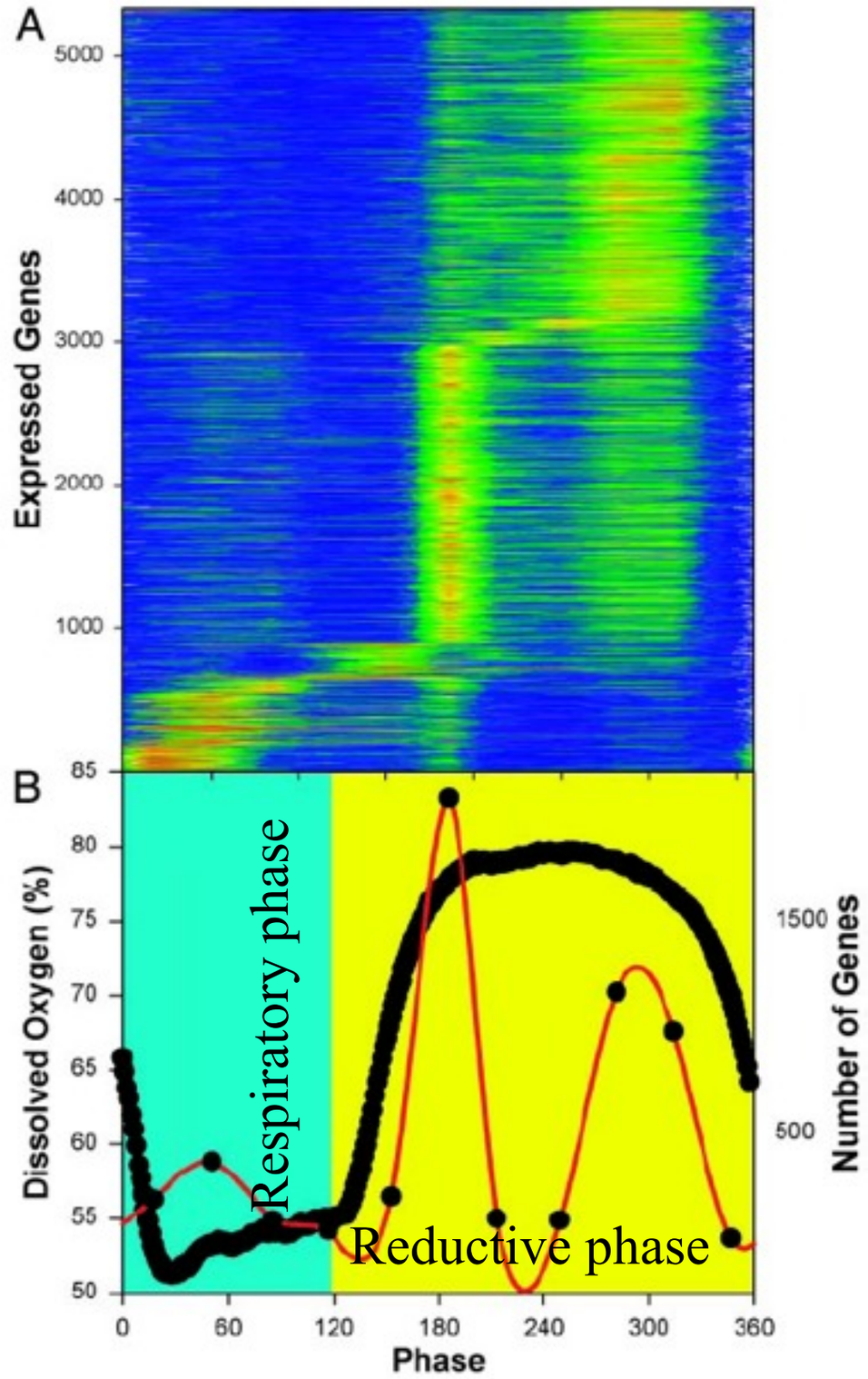
Klevecz et al, 2004

Respiratory cycle



5,329 expressed genes

Average peak-to-trough ratio ~2



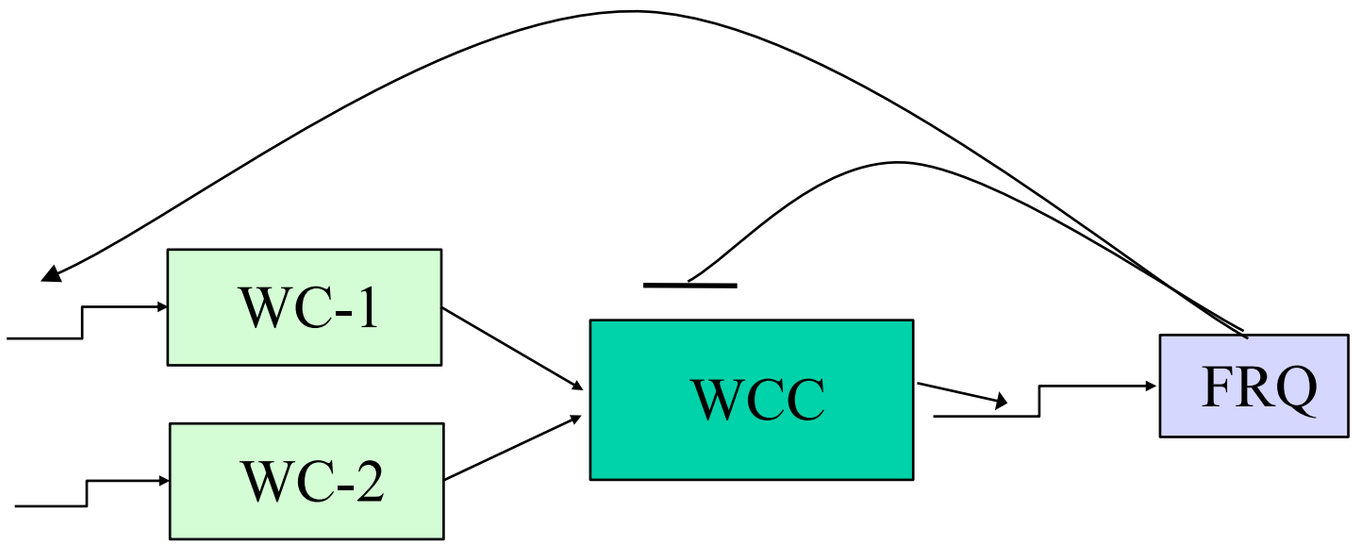
Circadian clock in *Neurospora crassa*

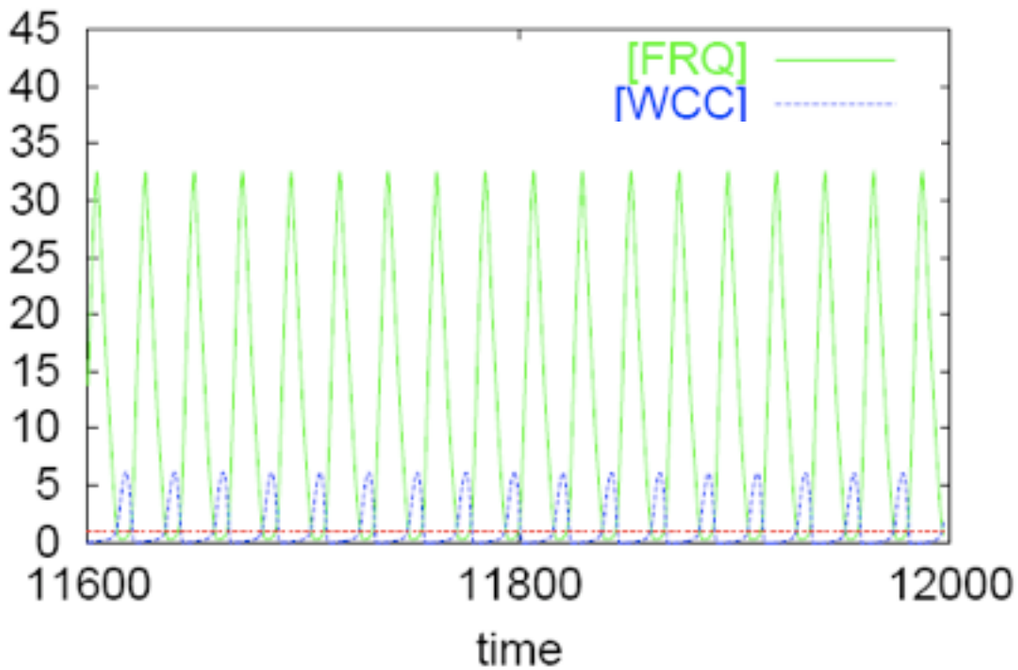
Stu Brody

$$\dot{f} = \frac{k_f w^2(t-\tau)}{1+K_w w^2(t-\tau)} - \gamma_f f - \kappa f w$$

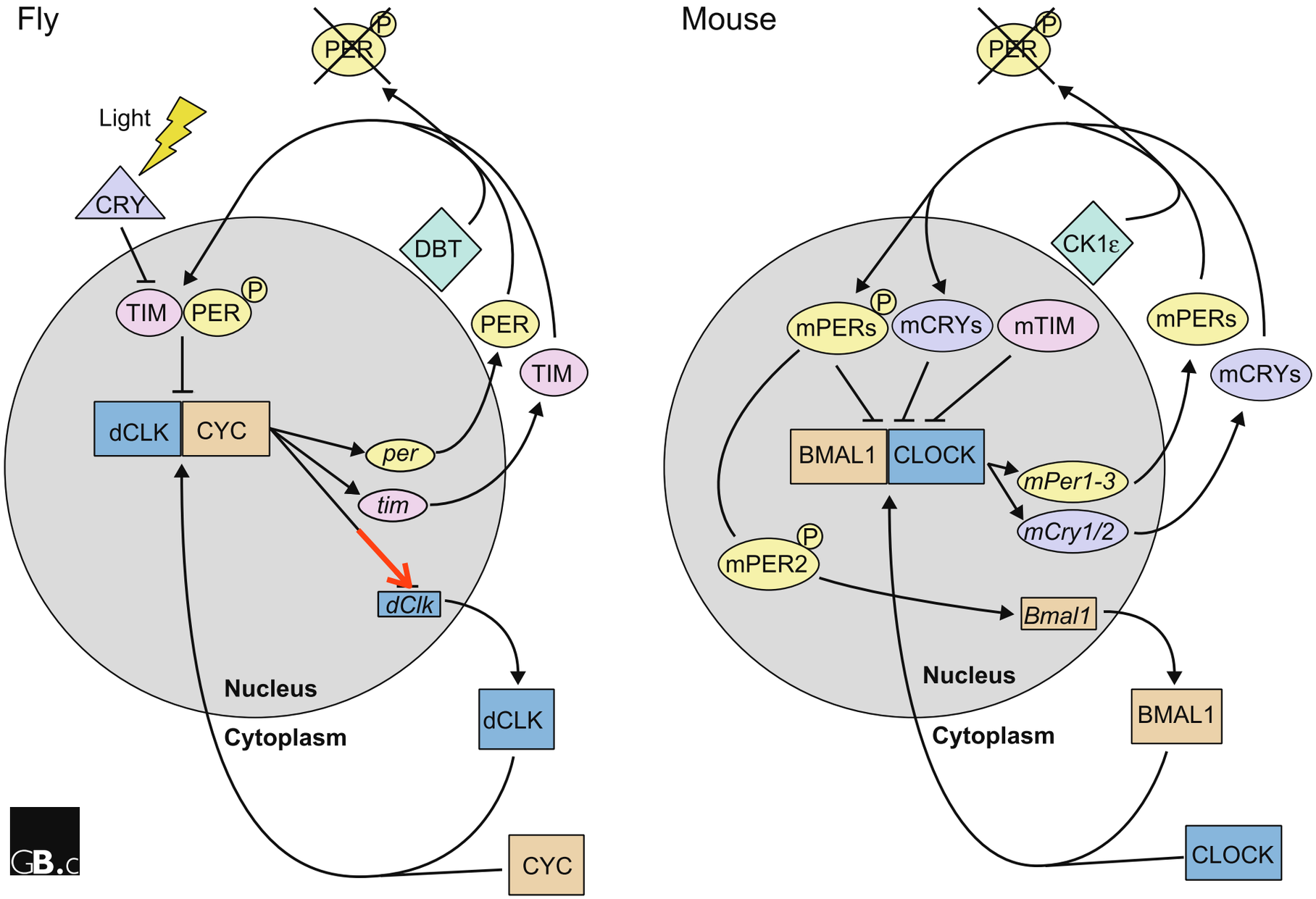
$$\dot{w} = \frac{k_f f^2(t-\tau)}{1+K_f f^2(t-\tau)} - \gamma_w w - \kappa f w$$

Sriram, Gopinathan, 2004





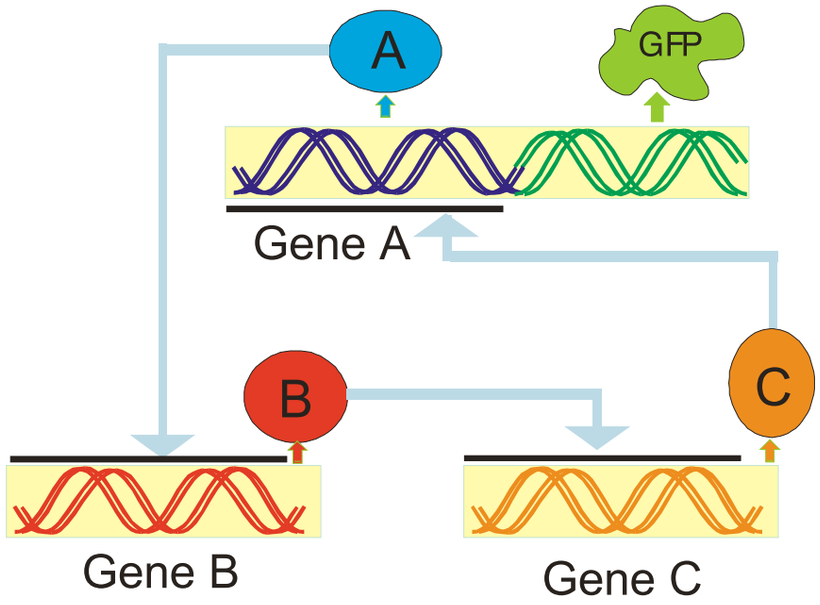
Circadian networks have similar regulatory mechanisms



http://www.biology-online.org

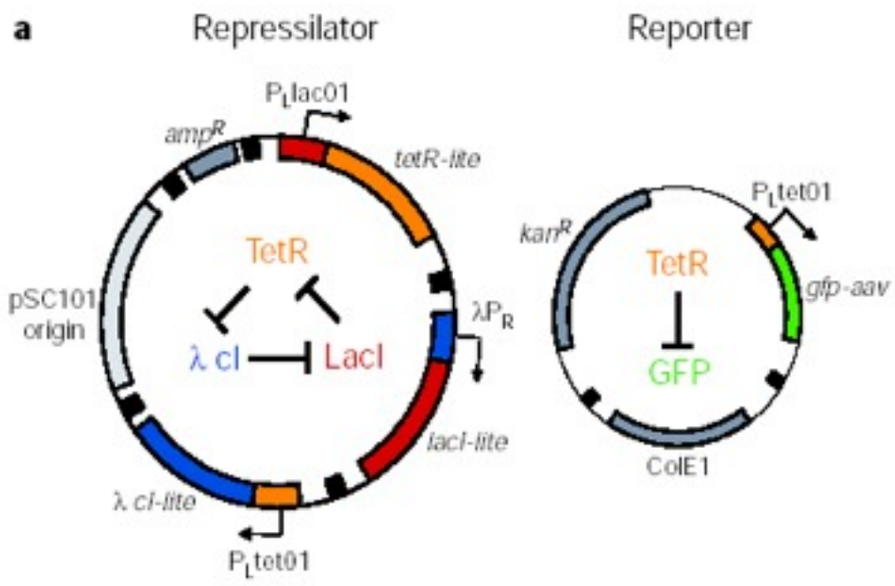
Interlocked positive and negative feedback loops

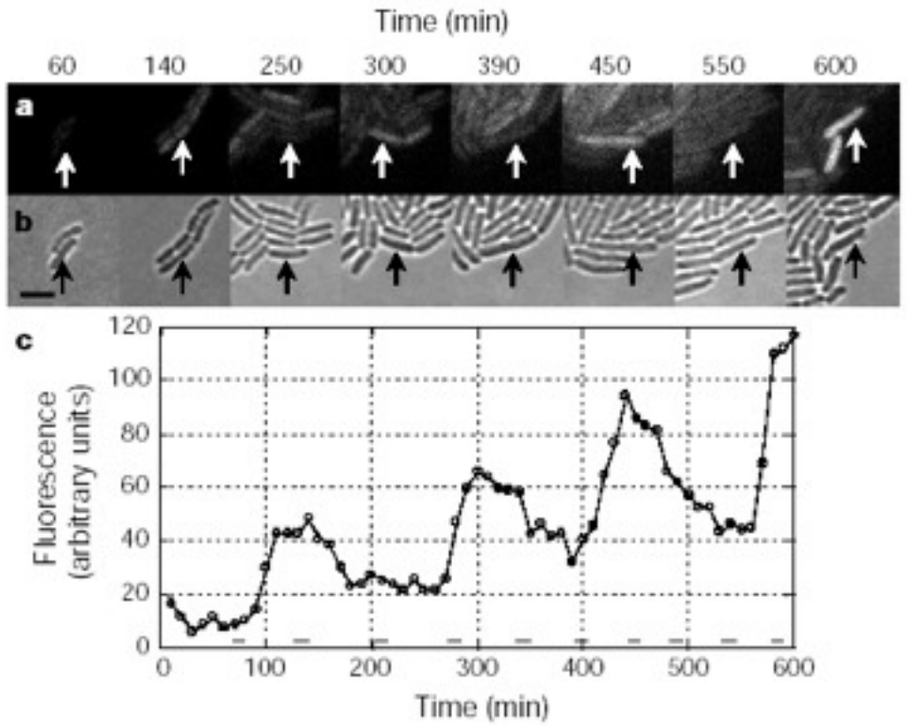
Repressilator: the first synthetic gene oscillator



$$\dot{m}_i = -m_i + \frac{\alpha}{1+p_i^n} + \alpha_0$$

$$\dot{p}_i = -\beta(p_i - m_i)$$

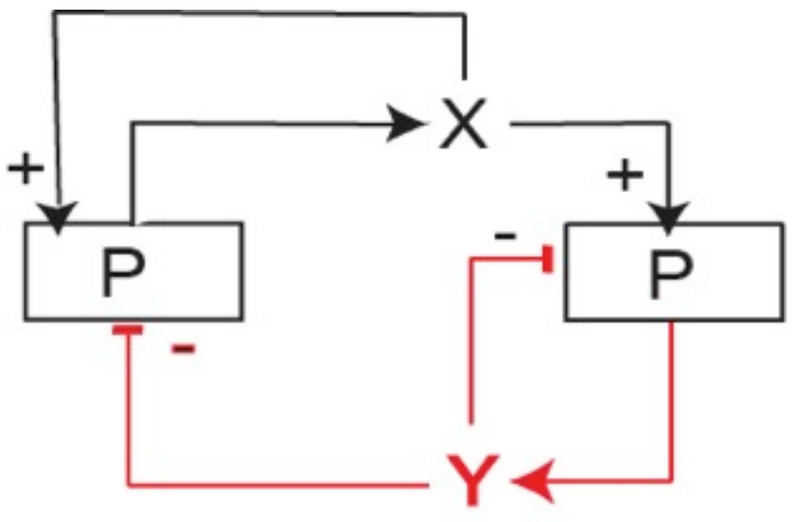




Elowitz and Leibler, *Science* 2001

Activated repression: A highly conserved motif in biology

(circadian networks, NFkB, galactose utilization,…)



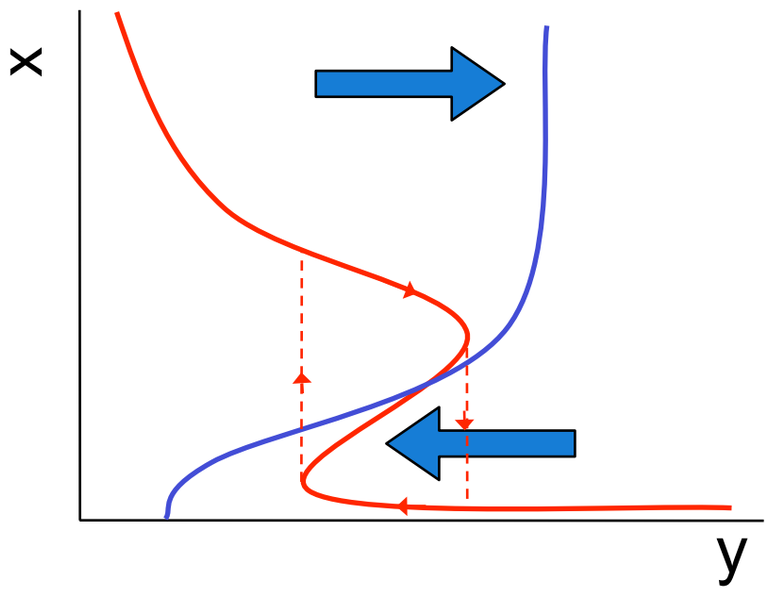
Equations for synthetic network
(derived from biochemical reactions)

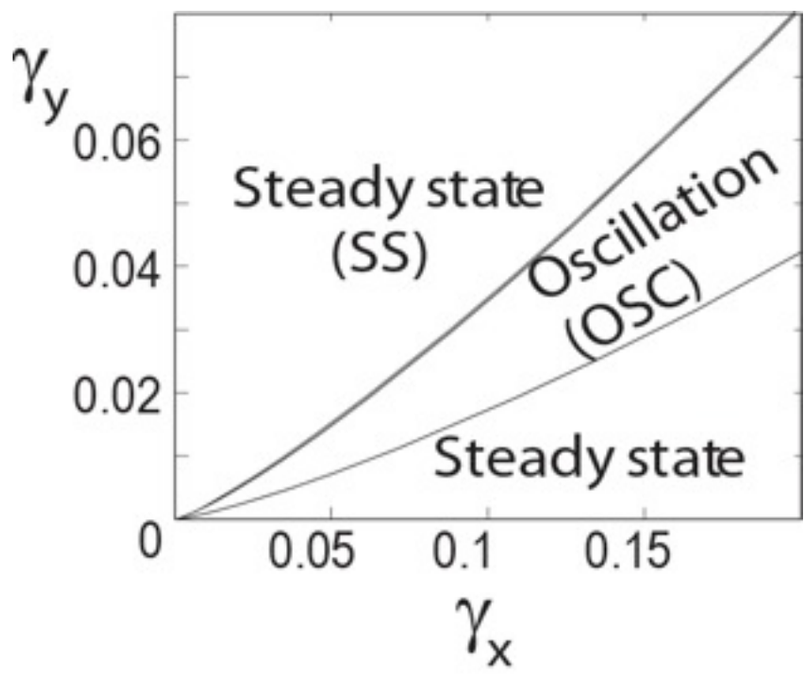
$$\frac{dx}{dt} = \frac{1 + x^2 + \alpha\sigma x^4}{(1 + x^2 + \sigma x^4)(1 + y^4)} - \gamma_x x\,,$$

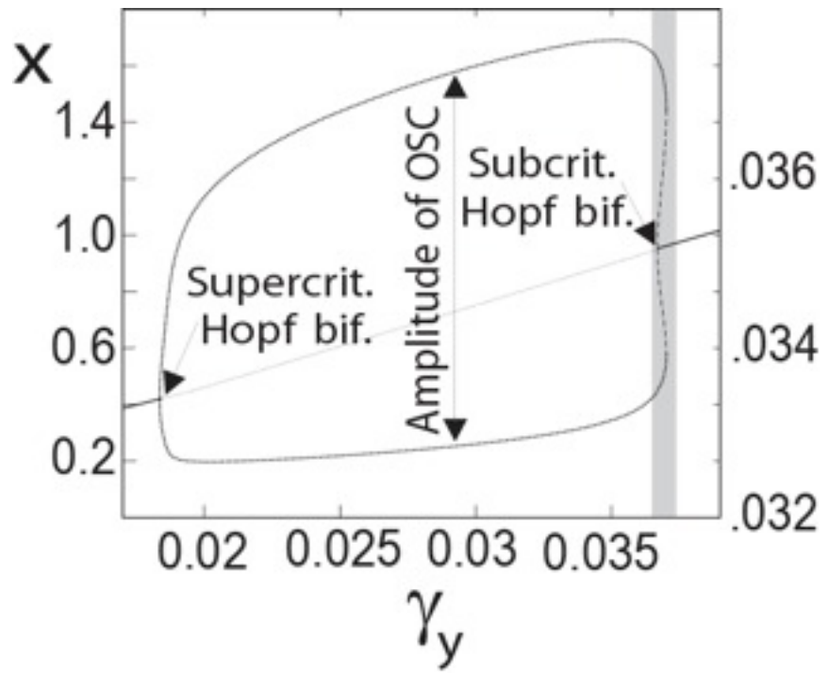
$$\tau_y \frac{dy}{dt} = \frac{1 + x^2 + \alpha\sigma x^4}{(1 + x^2 + \sigma x^4)(1 + y^4)} - \gamma_y y\,.$$

(Hasty et al, PRL 2002)

FitzHugh-Nagumo-like dynamics:

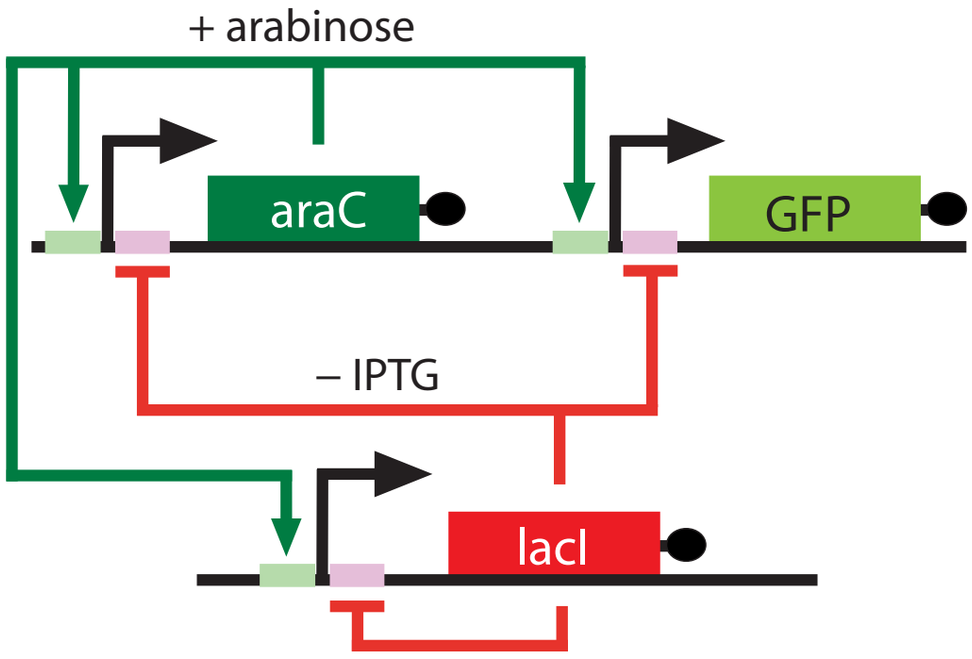


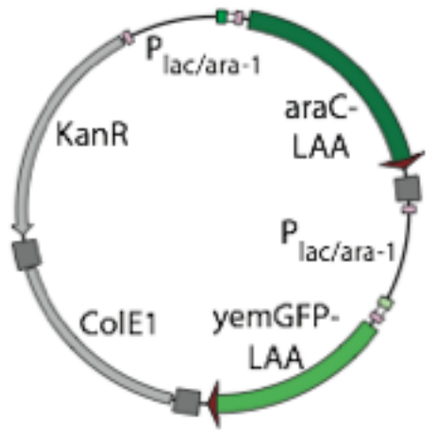




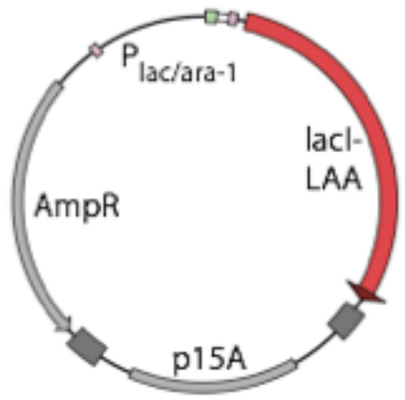
Synthetic gene oscillator in in *E.coli*

Stricker *et al.*, Nature,456(7221):516-9 (2008).





activator/reporter
plasmid
(pJS167)



repressor
plasmid
(pJS169)

Activator: araC - up-regulates the promoter in the presence of arabinose.

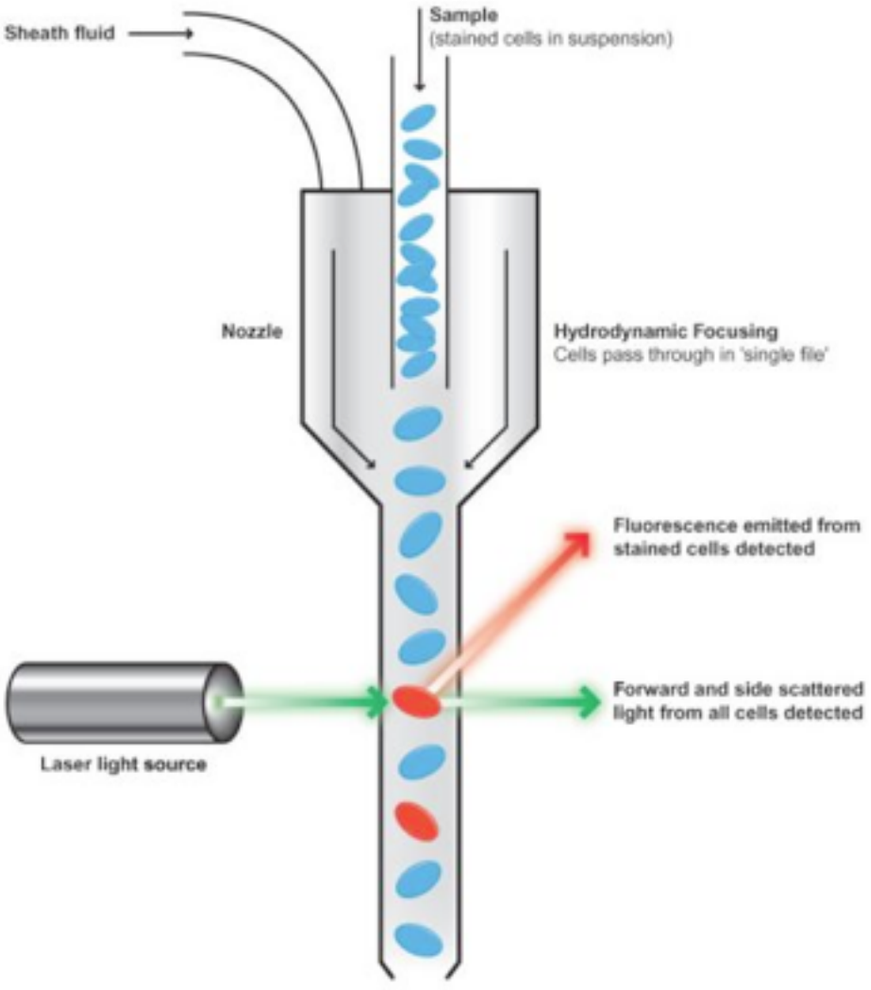
Repressor: lacI - down-regulates the promoter in the absence of IPTG.

Promoter: The hybrid Plac/ara-1 promoter drove each component. (Lutz and Bejard, Nucleic Acids Res. **25**, 1203-1210 (1997)).

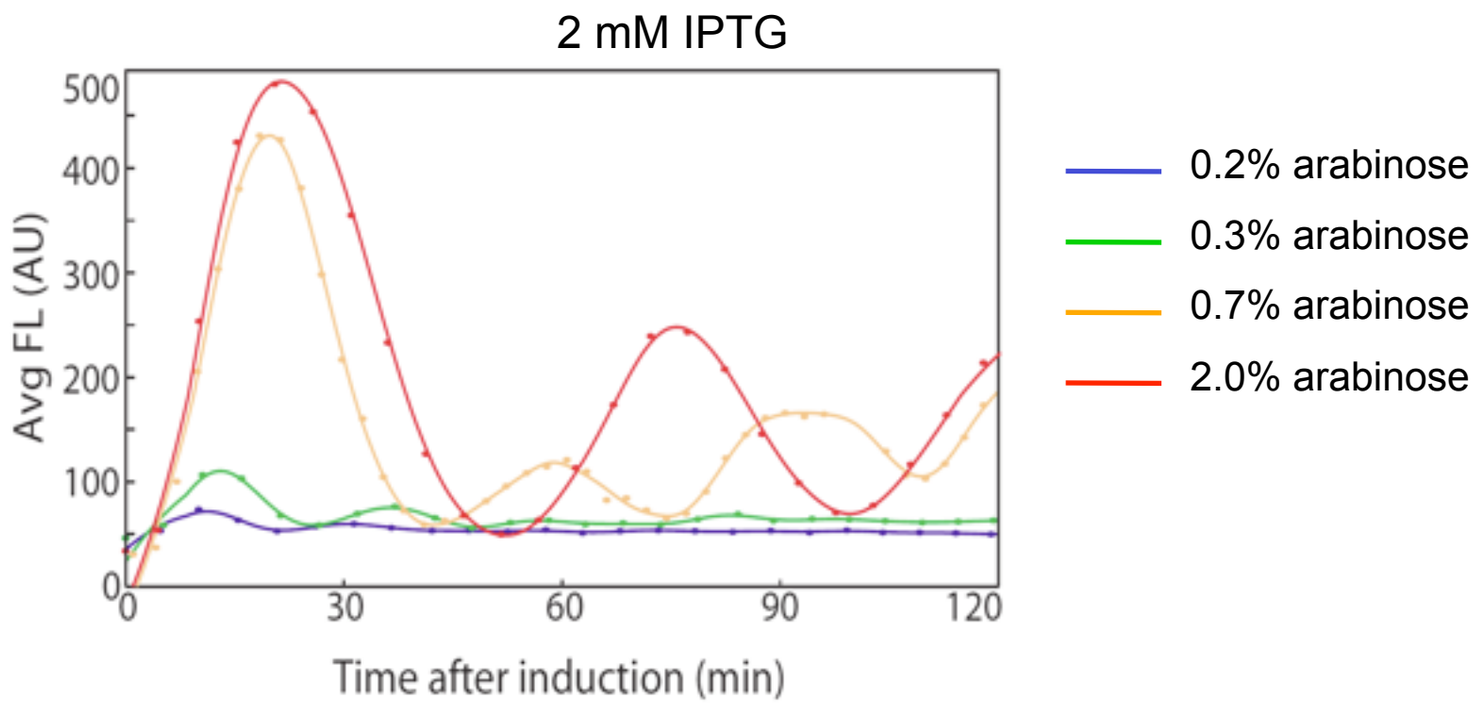
Degradation: The same ssrA tags were added to each gene to increase temporal resolution (AANDENYALAA).

- Two experimental “knobs”: Arabinose required for activator binding and IPTG prohibits repressor binding

Flow cytometry experiments



time series of fluorescence in initially synchronized batch culture of cells

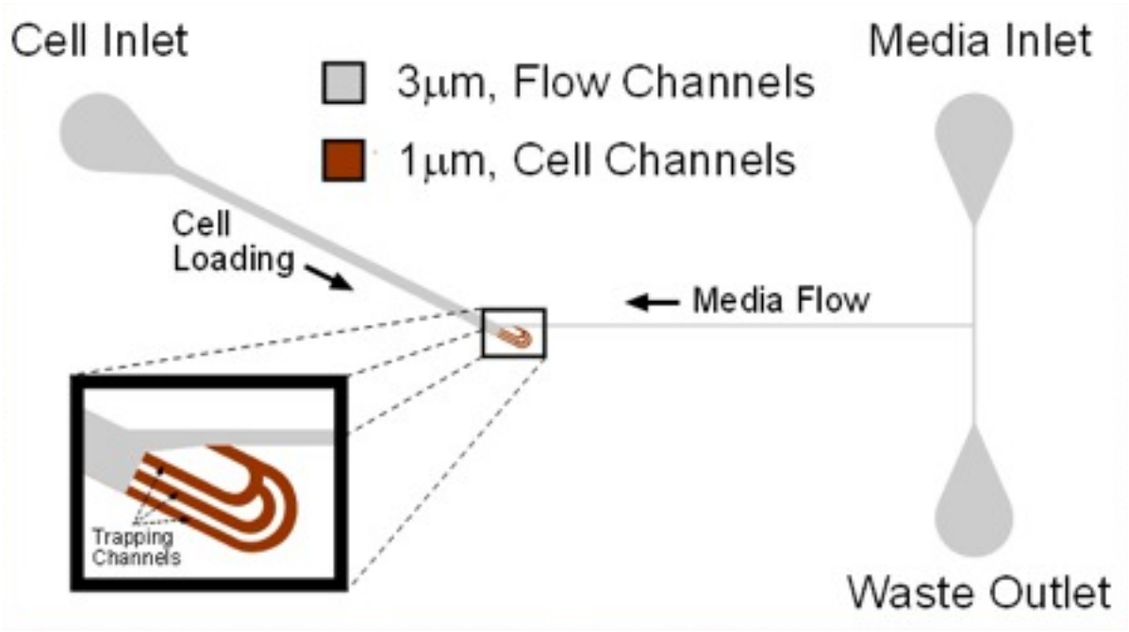


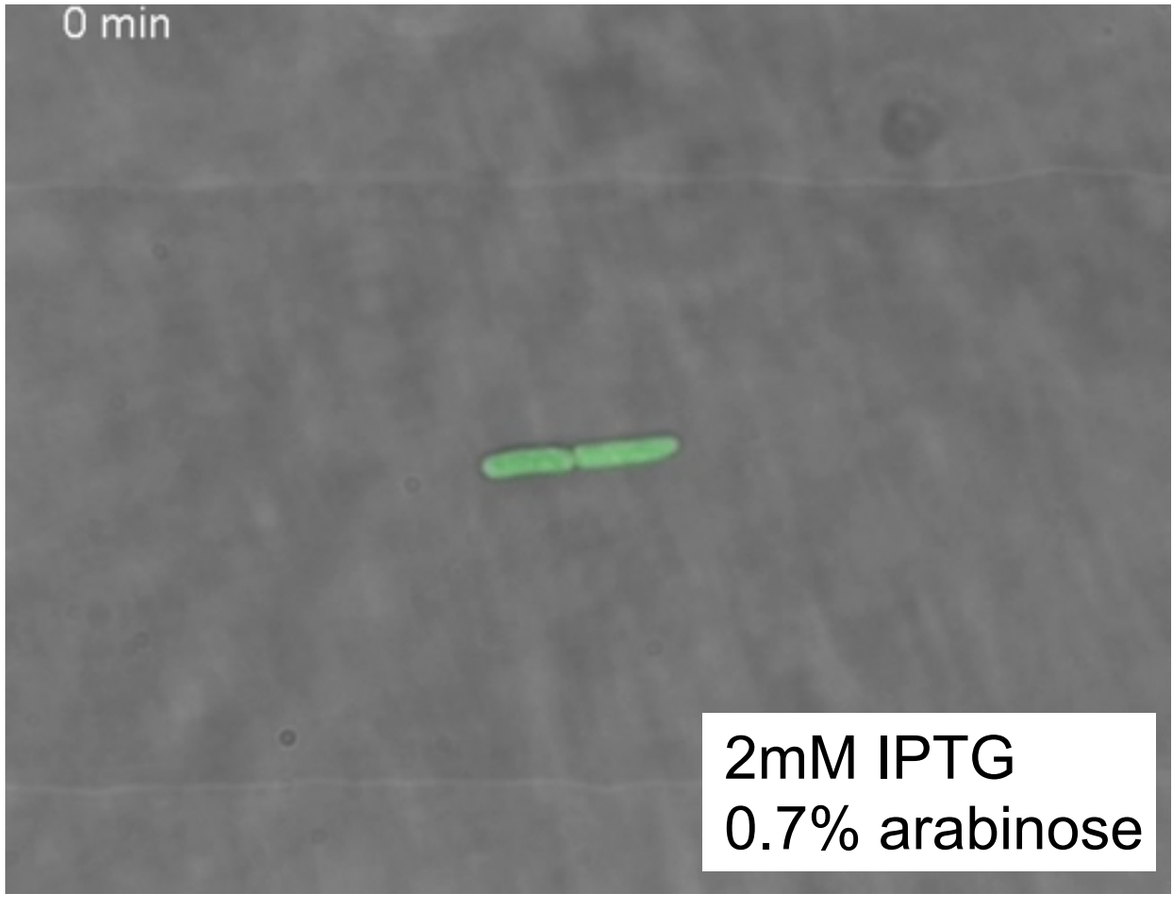
What is it: oscillations stop
…or desynchronize?

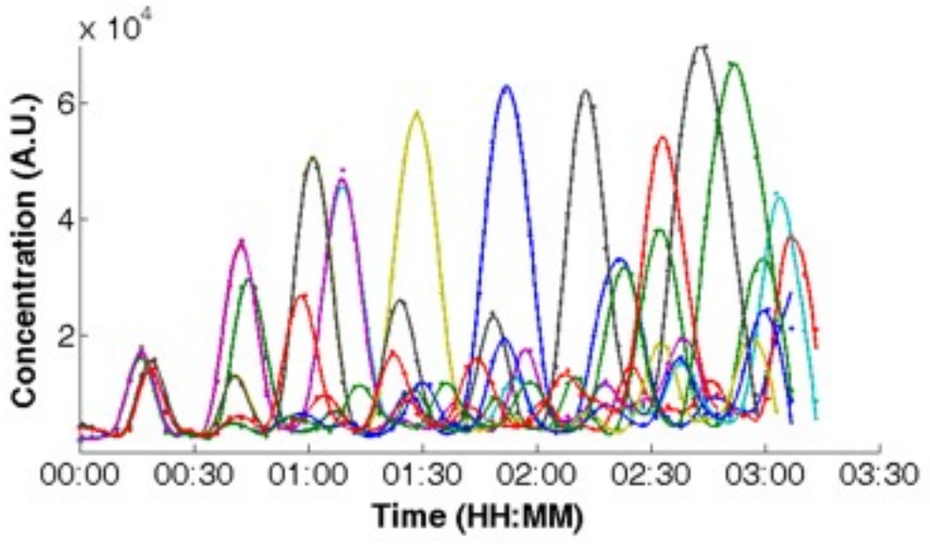
Single cell experiments

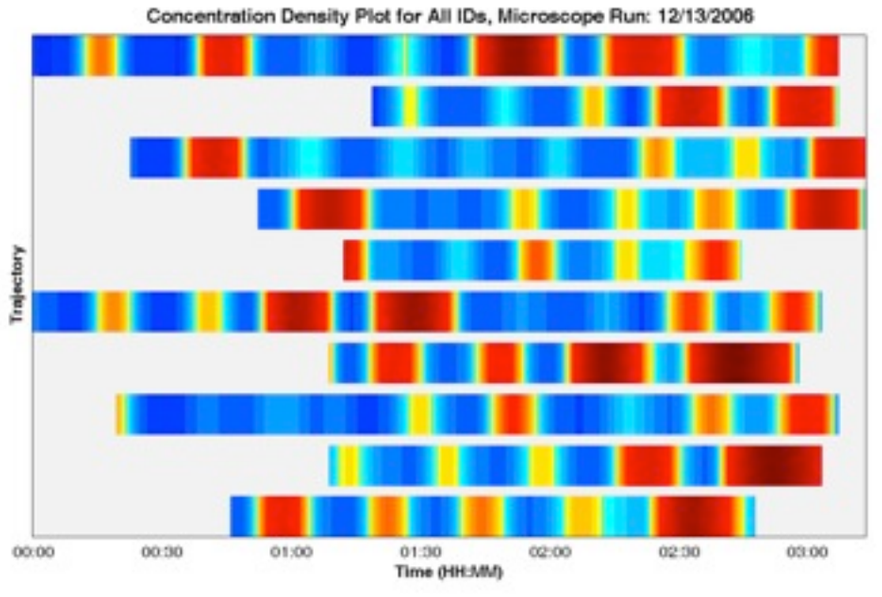
Stricker *et al.*, Nature,456(7221):516-9 (2008).

Microfluidic chip



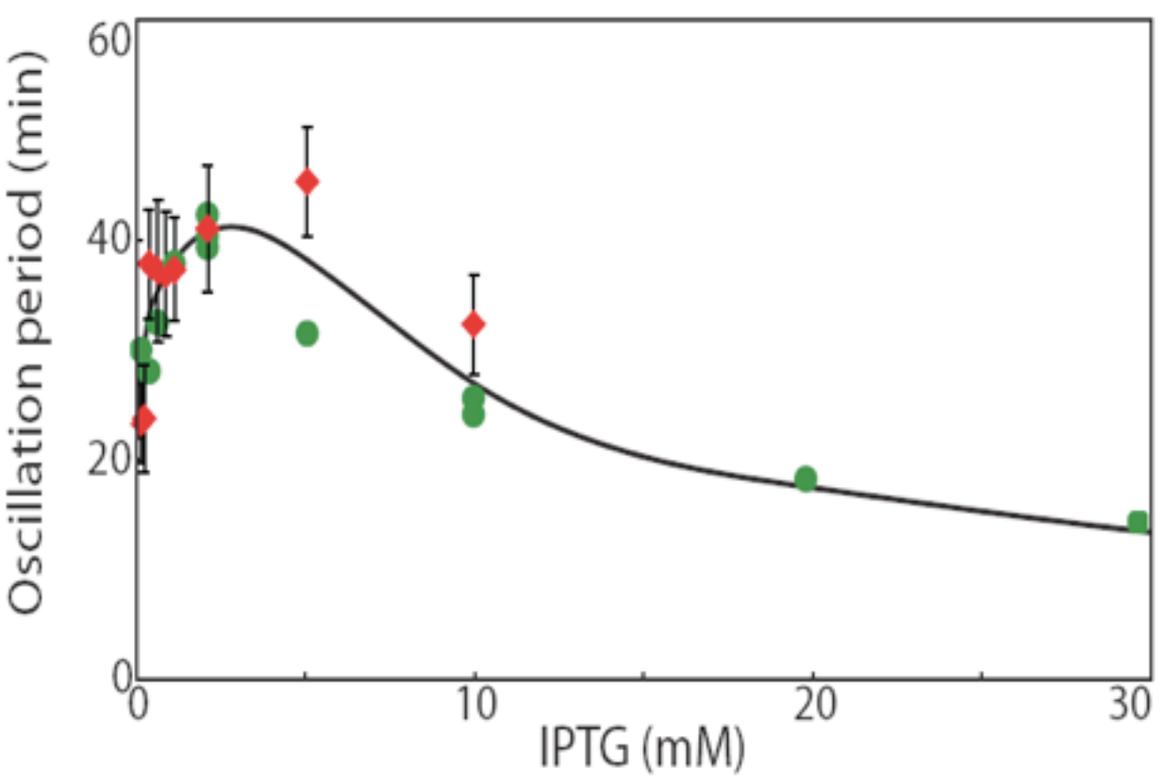


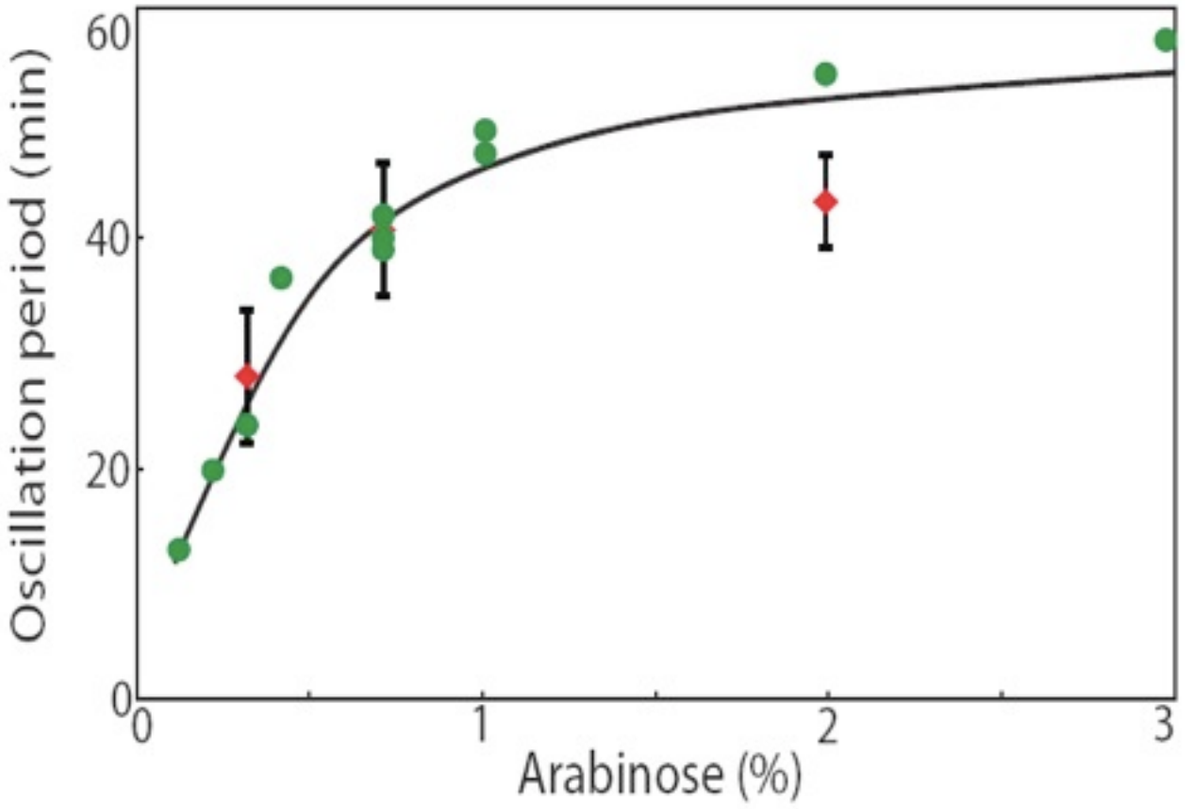




Tunable and robust oscillations

Stricker *et al.*, Nature,456(7221):516-9 (2008).





- Large amplitude oscillations (from full expression to zero)
- More than 95% of all cells oscillate throughout the runs

A conceptually similar synthetic oscillator in mammalian cells:
Tigges *et al.* A tunable synthetic mammalian oscillator, Nature 457, 309-312 (2009)

Bacterial oscillator: A detailed model

Stricker et al., Nature,456(7221):516-9 (2008).

$$P_{0,0}^{a/r} \xrightarrow{b_{a/r}} P_{0,0}^{a/r} + m_{a/r}$$

$$P^{a/r} \xrightarrow{\alpha b_{a/r}} P^{a/r} + m_{a/r}$$

$$P_{0,j}^{a/r} + a_2 \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} P_{1,j}^{a/r}$$

$$P_{i,0}^{a/r} + r_4 \underset{k_{-r}}{\overset{2k_r}{\rightleftharpoons}} P_{i,1}^{a/r}$$

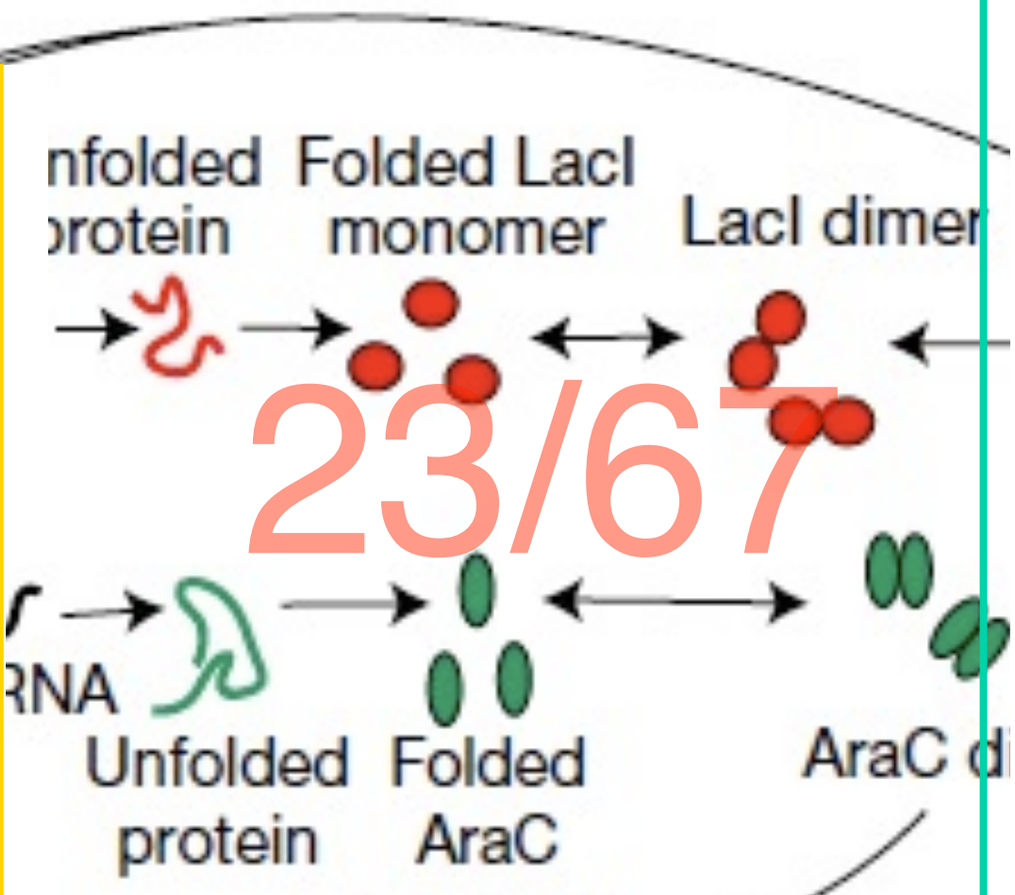
$$P_{i,1}^{a/r} + r_4 \underset{2k_{-r}}{\overset{k_r}{\rightleftharpoons}} P_{i,2}^{a/r}$$

$$P_{1,2}^{a/r} \xrightarrow{k_l} P_{L,2}^{a/r} + a_2$$

$$P_{0,2}^{a/r} \xrightarrow{k_l} P_{L,2}^{a/r}$$

$$P_{L,0}^{a/r} \xrightarrow{k_{ul}} P_{0,0}^{a/r},$$

Regulation



Transcription/translation

$$k_r = k_{-r}\left(\left(C_r^{max} - C_r^{min}\right)\frac{1}{1+\left(\frac{[IPTG]}{k_{r1}}\right)^{b_1}} + C_r^{min}\right)$$

$$k_a = k_{-a}\left(\left(C_a^{max} - C_a^{min}\right)\cdot\frac{[ara]^{c_1}}{k_{a1}^{c_1}+[ara]^{c_1}}\cdot\frac{1}{1+\left(\frac{[IPTG]}{k_{r2}}\right)^{b_2}} + C_a^{min}\right)$$

$i \in \{0,1\}$
$j \in \{0,1,2\}$

$$m_{a/r} \xrightarrow{d_{a/r}} \emptyset$$

$$a_{uf} \xrightarrow{\lambda f(X)} \emptyset$$

$$r_{uf} \xrightarrow{f(X)} \emptyset$$

$$a \xrightarrow{\lambda f(X)} \emptyset$$

$$r \xrightarrow{f(X)} \emptyset$$

$$a_2 \xrightarrow{\lambda f(X)} \emptyset$$

$$r_2 \xrightarrow{f(X)} \emptyset$$

$$r_4 \xrightarrow{f(X)} \emptyset$$

$$P_{1,j}^{a,r} \xrightarrow{f(X)} P_{0,j}^{a,r}$$

$$P_{i,1}^{a,r} \xrightarrow{f(X)} P_{i,0}^{a,r}$$

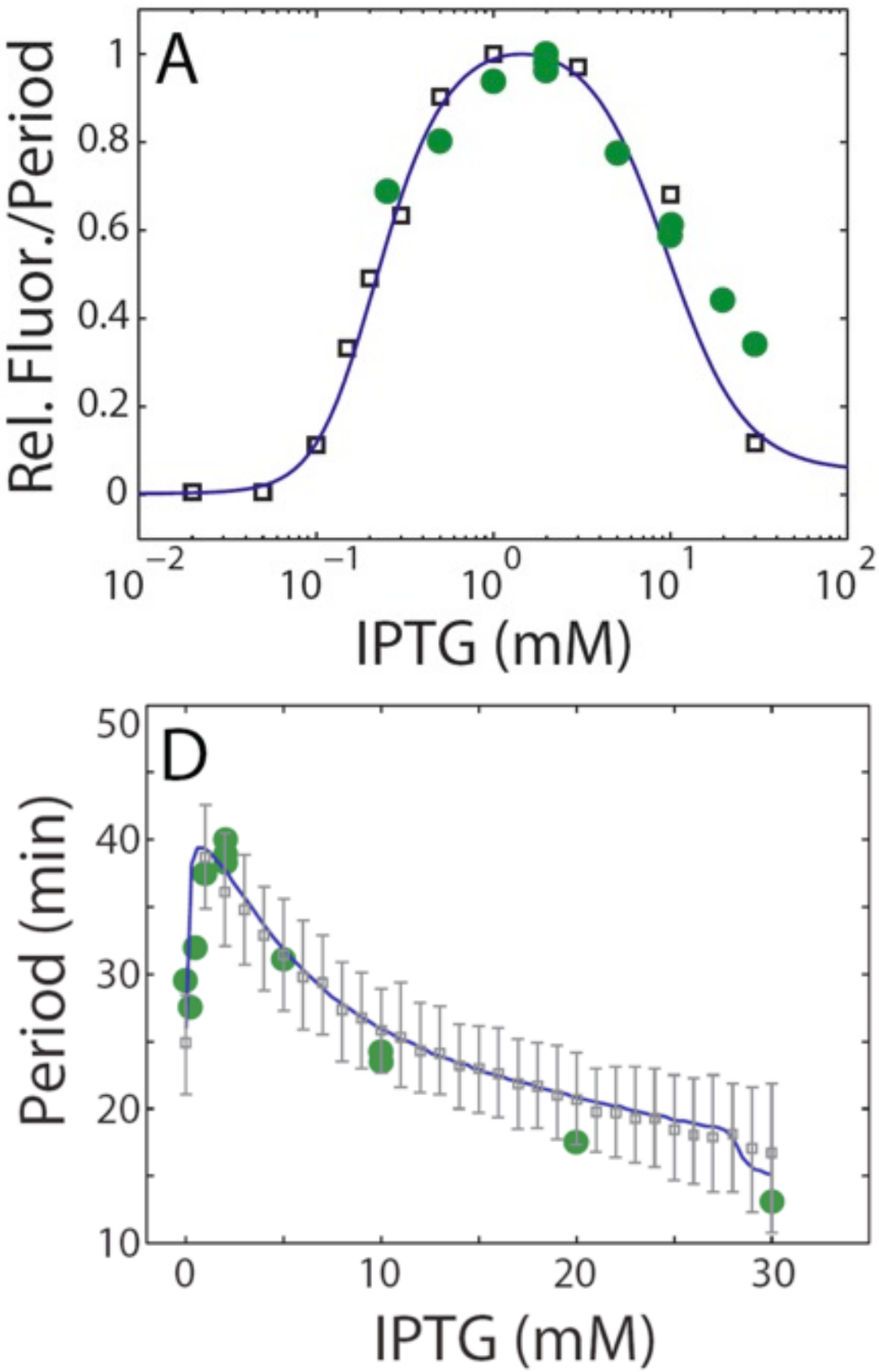
$$P_{i,2}^{a,r} \xrightarrow{2f(X)} P_{i,1}^{a,r}$$

$$P_{L,2}^{a,r} \xrightarrow{2\epsilon f(X)} P_{L,1}^{a,r}$$

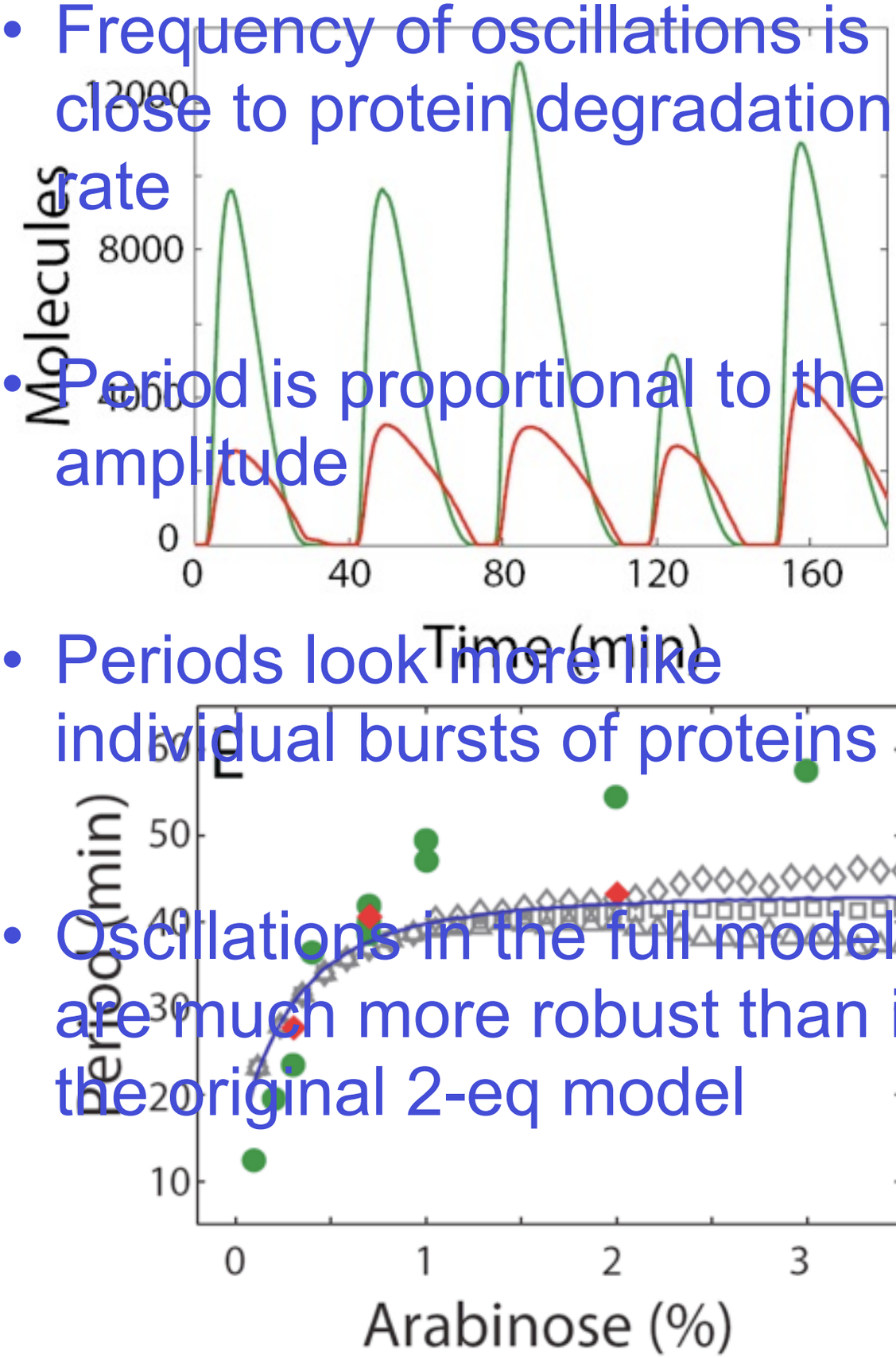
$$P_{L,1}^{a,r} \xrightarrow{\epsilon f(X)} P_{L,0}^{a,r}$$

Enzymatic decay $f(X) = \frac{\gamma}{c_e + X}$

Bacterial oscillator: simulation results

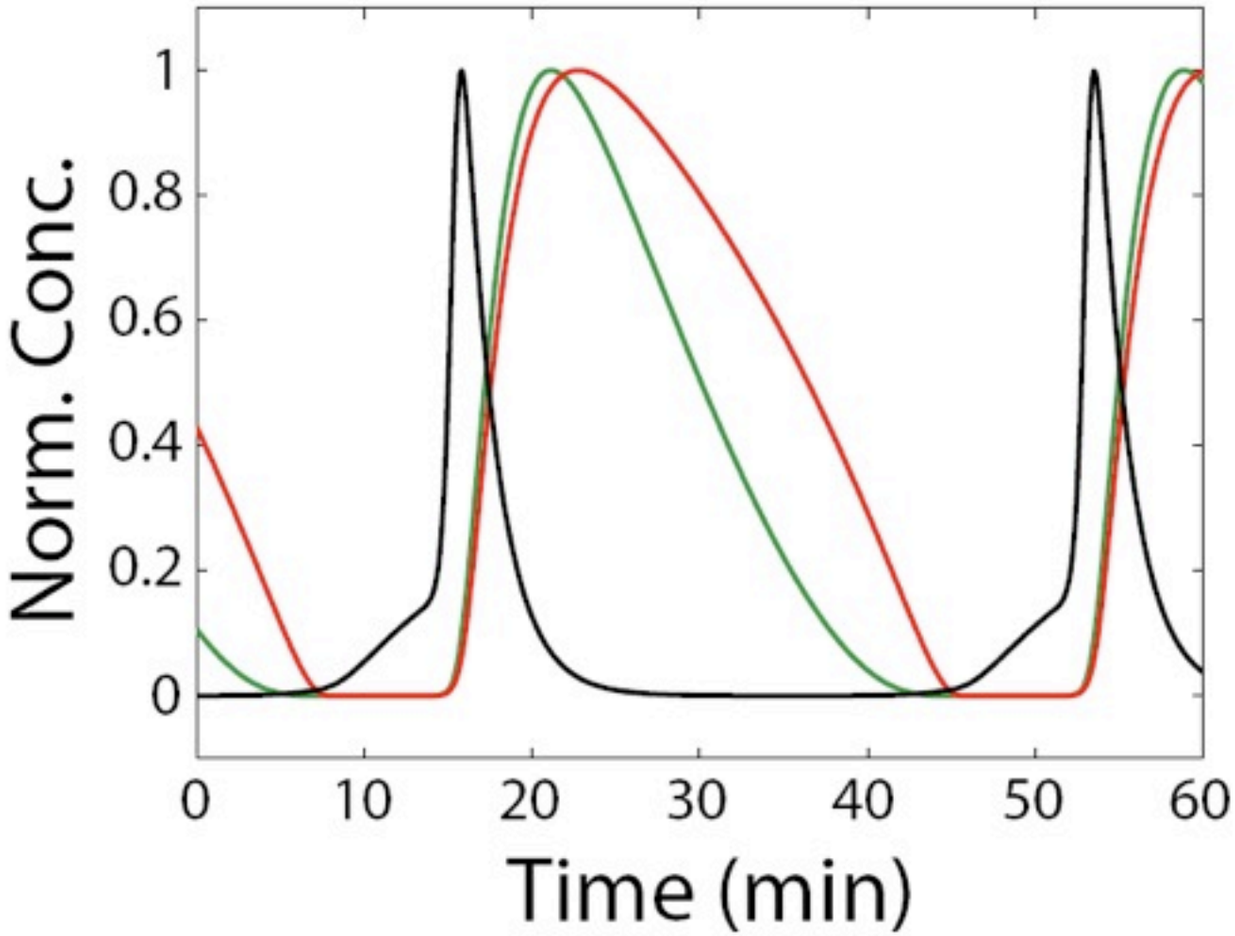


- Frequency of oscillations is close to protein degradation rate
- Period is proportional to the amplitude



- Periods look more like individual bursts of proteins
- Oscillations in the full model are much more robust than in the original 2-eq model

Mechanism of oscillations



Activator/repressor mRNA

activator protein

repressor protein

A burst of mRNAs produces activator and then repressor proteins which slowly degrade until the promoters are free of repressor and the next burst ensues. A small *delay* allows the circuit to produce enough mRNA before repressor shuts down transcription.

degrade-and-fire model?

Explicit delay model

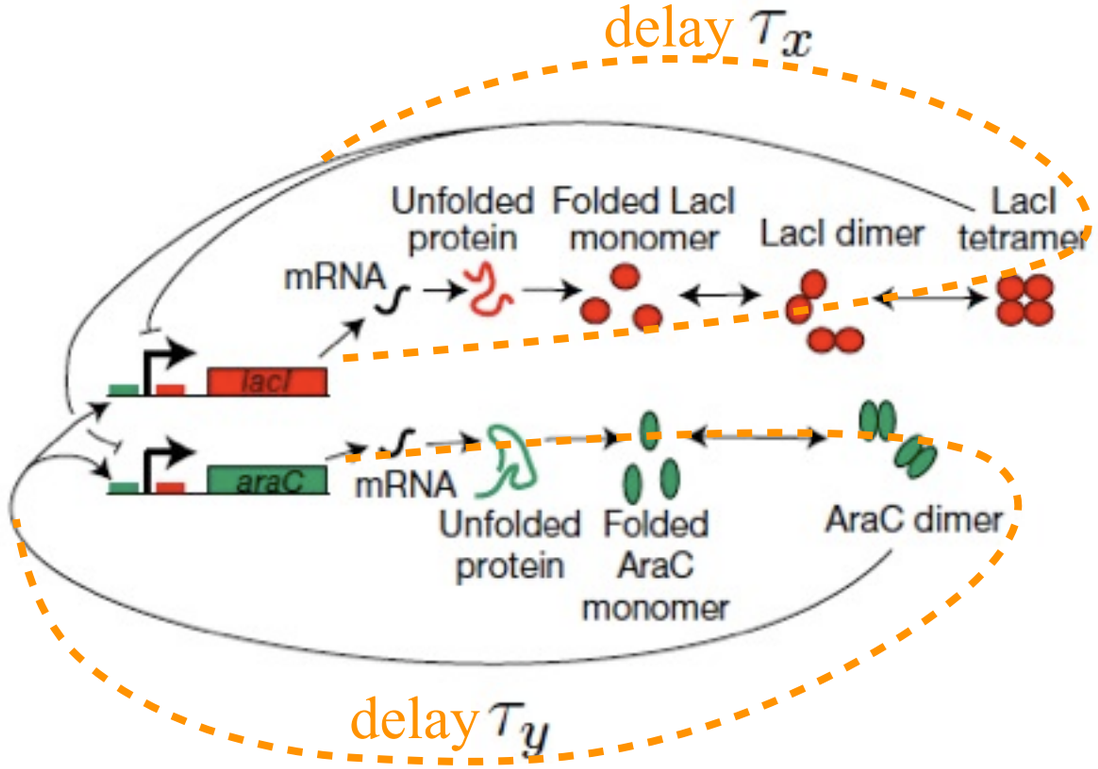
replace chains of interactions with a single effective delay in feedback

lumps many potentially unknown parameters into a few meaningful parameters (identify ignorance)

makes mechanism more transparent

amendable to analytic treatment

faster simulations using delay approximation

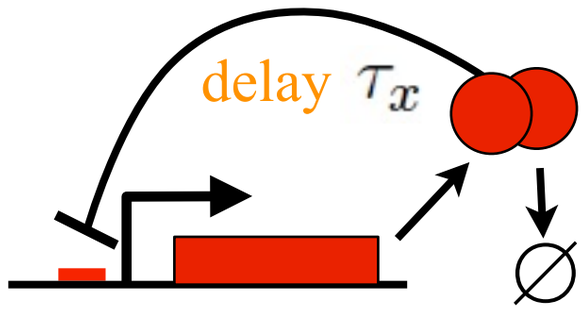


$$\frac{dx}{dt} = \frac{\alpha(1 + f y_{\tau_y}^2 / C_{0y}^2)}{(1 + x_{\tau_x}^2 / C_{0x}^2)(1 + y_{\tau_y}^2 / C_y^2)} - \frac{\delta_x x}{C_{1x} + x}$$

$$\frac{dy}{dt} = \frac{\alpha(1 + f y_{\tau_y}^2 / C_{0y}^2)}{(1 + x_{\tau_x}^2 / C_{0x}^2)(1 + y_{\tau_y}^2 / C_y^2)} - \frac{\delta_y y}{C_{1y} + y}$$

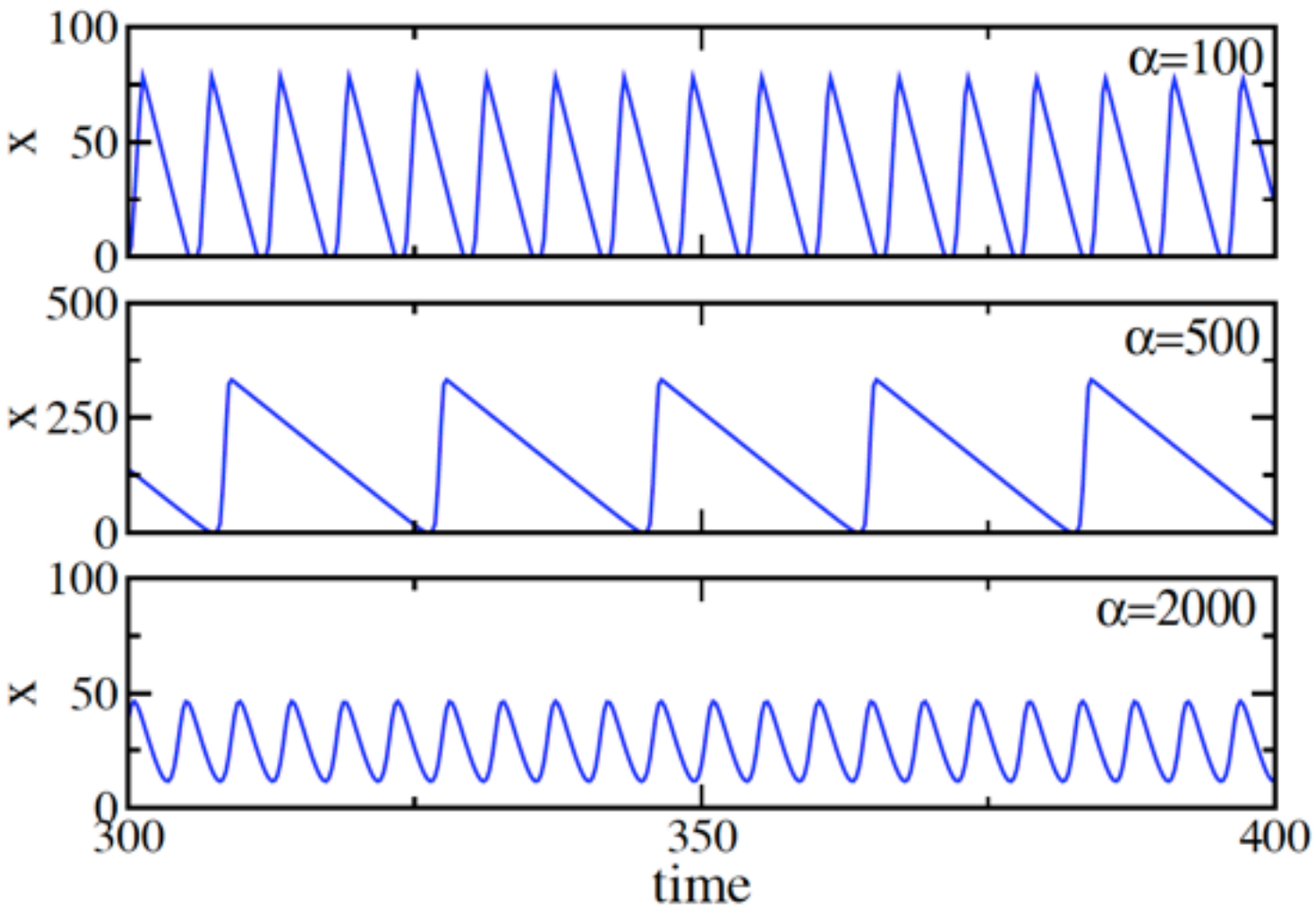
Delayed auto-repression: one gene system

Mather et al, PRL 102, 068105 (2009)

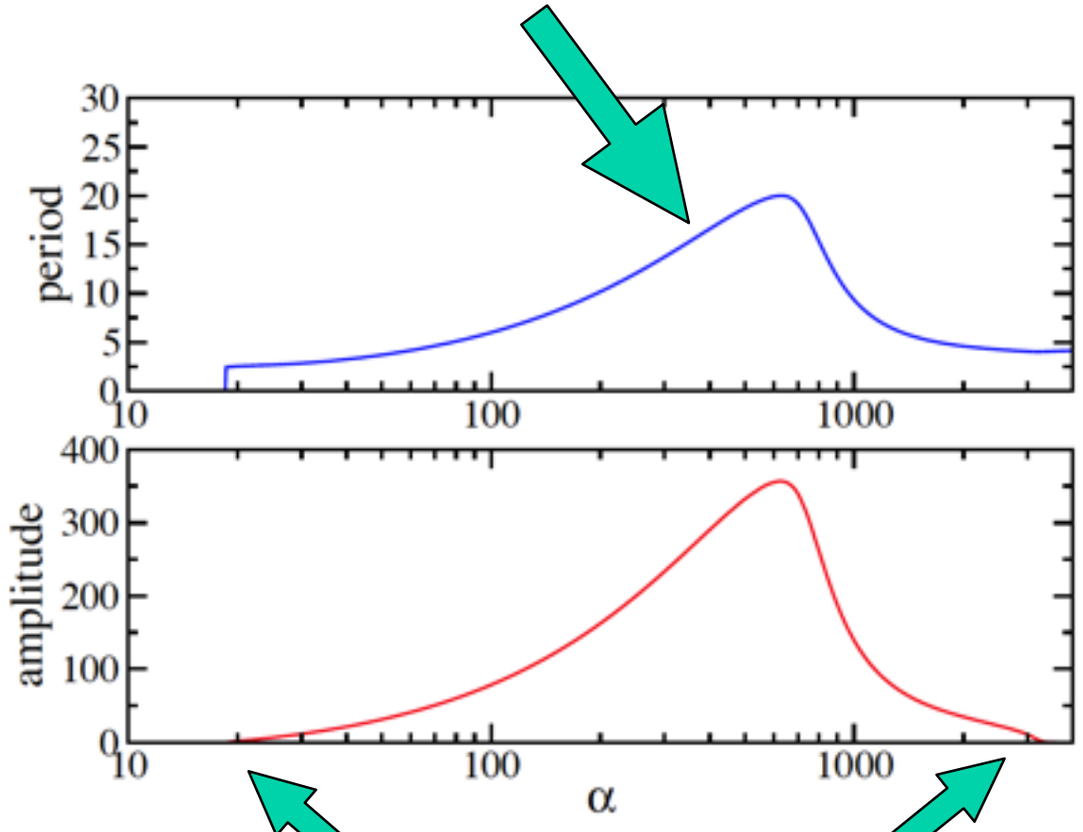


$$\dot{x}(t) = \frac{\alpha}{\left(1+\frac{x(t-\tau)}{C_0}\right)^2} - \delta\frac{x(t)}{C_1+x(t)}$$

$\tau = 1; \delta = 20; C_0 = 2; C_1 = 0.1$



Degrade-and-fire regime

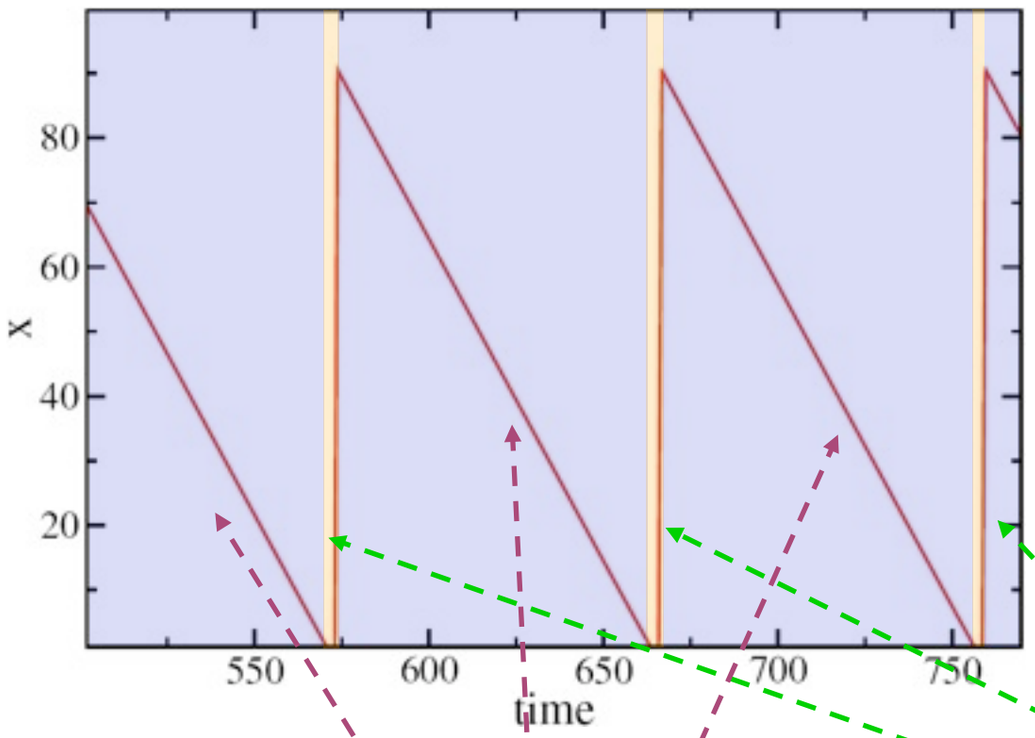


Hopf bifurcations

Delayed auto-repression: degrade-and-fire model

Mather et al, PRL 102, 068105 (2009)

$\tau = 1; \alpha = 100; C_0 = C_1 = 0.01; \delta = 1$



$$\dot{x}(t) = \frac{\alpha}{\left(1 + \frac{x(t-\tau)}{C_0}\right)^2} - \delta \frac{x(t)}{C_1 + x(t)}$$

α large; C_0, C_1 small

Fast production

$$\dot{x}_p(t) = \frac{\alpha}{\left(1 + \frac{x_p(t-\tau)}{C_0}\right)^2}$$

Slow degradation

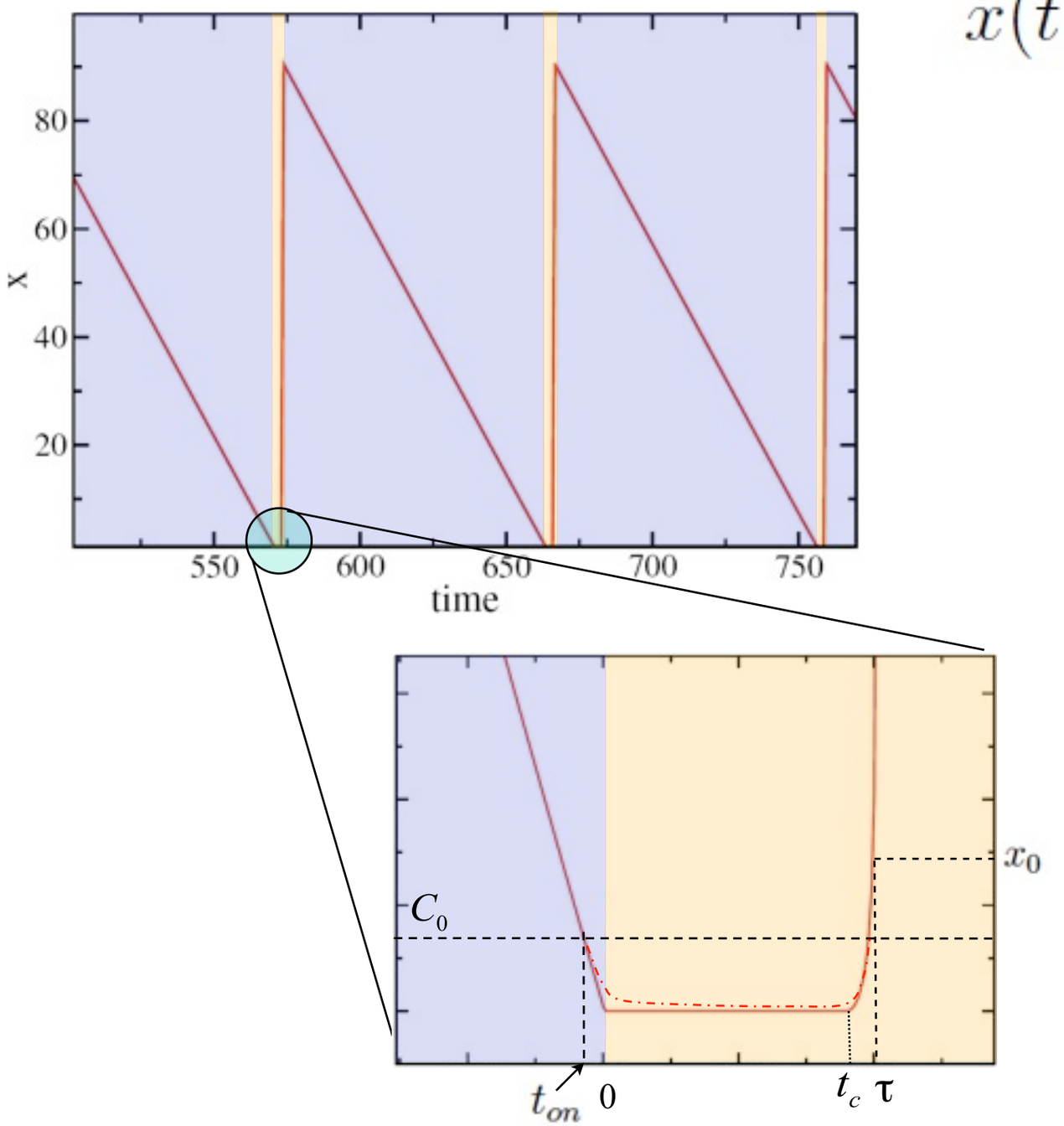
$$\dot{x}_d(t) = -\delta \frac{x_d(t)}{C_1 + x_d(t)}$$

$$x_d(t) = C_1 \mathcal{L}\left(e^{-\delta(t-t_0)}\right)$$

- Two time scales: relaxation oscillations

Zeroth-order degradation

$\tau = 1; \alpha = 100; C_0 = 0.01; \delta = 1$



$$\dot{x}(t) = \frac{\alpha}{\left(1 + \frac{x(t-\tau)}{C_0}\right)^2} - \delta\Theta(x(t))$$

Degradation

$$\dot{x}_d(t) = -\delta\ \Theta(x_d(t))$$

$$x_d(t) = -\delta t; \quad t < 0$$

Production

$$\dot{x}_p(t) = \frac{\alpha}{\left(1 - \frac{\delta(t-\tau)}{C_0}\right)^2} \qquad 0 < t < \tau$$

$$x_p(t) = 0; \quad 0 < t < t_c$$

$$\frac{\alpha}{\left(1 - \frac{\delta(t_c-\tau)}{C_0}\right)^2} = \delta \quad \Rightarrow \quad t_c = \tau - C_0\left(\sqrt{\frac{\alpha}{\delta}} - 1\right)$$

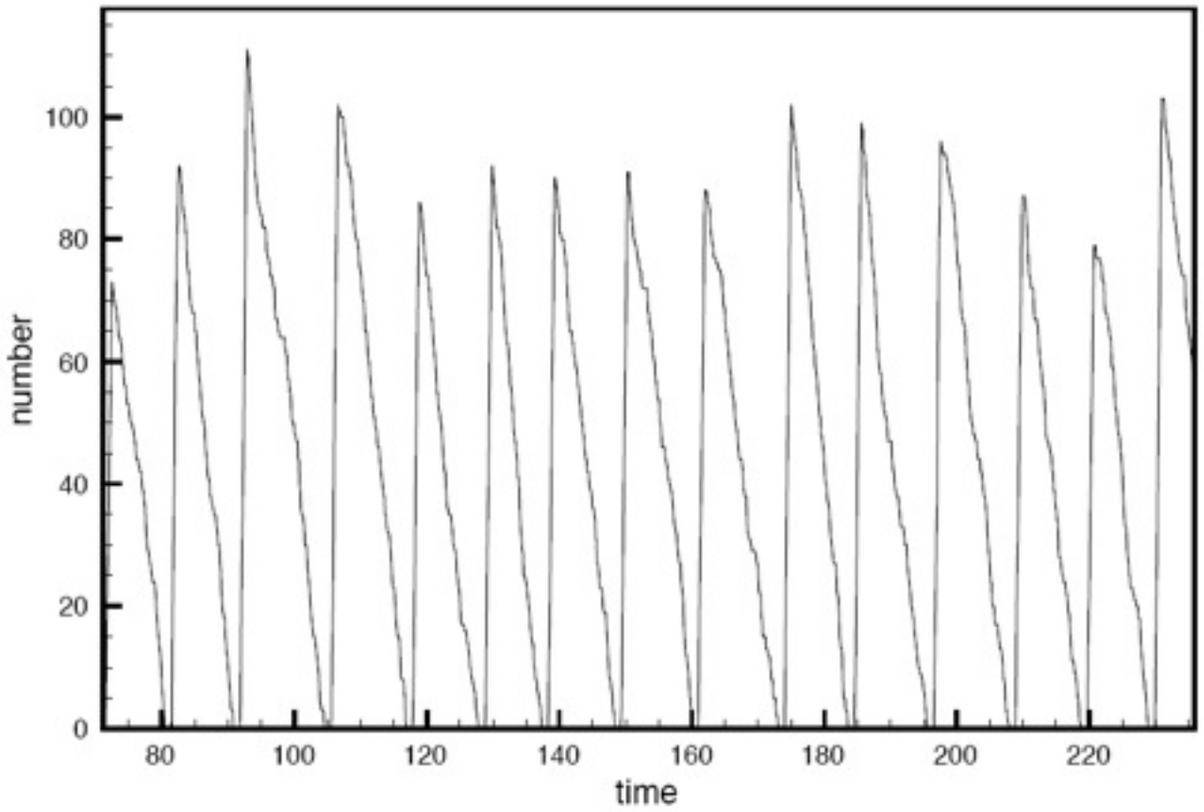
$$x_p(t) = x_0 + \alpha(t-\tau); \quad \tau < t < \tau + t_c$$

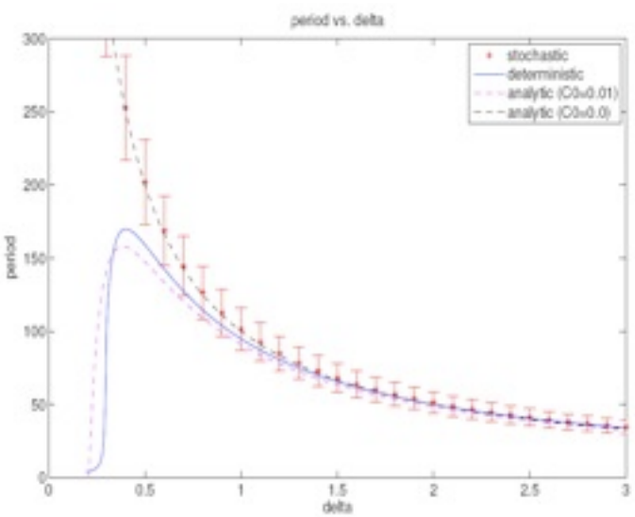
Maximum: $x_{\max} \approx \alpha t_c \approx \alpha\tau$

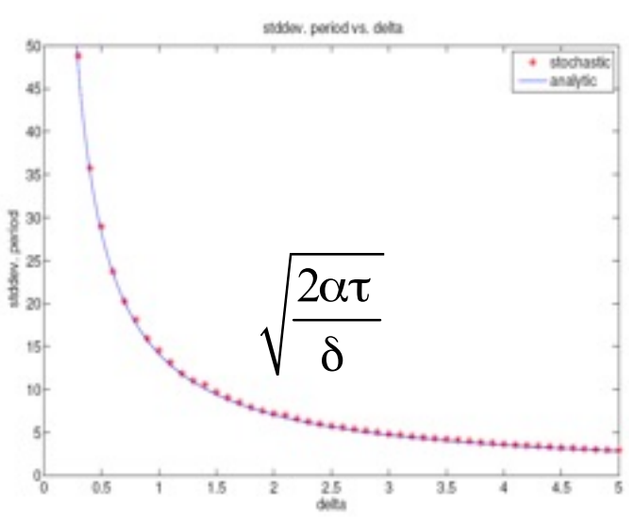
Period: $T \approx \alpha t_c/\delta + \tau + t_c \approx \alpha\tau/\delta$

Zeroth-order degradation: stochastic model

$$\tau = 1; \alpha = 100; C_0 = 0.01; \delta = 1$$







$$\sqrt{\frac{2\alpha\tau}{\delta}}$$

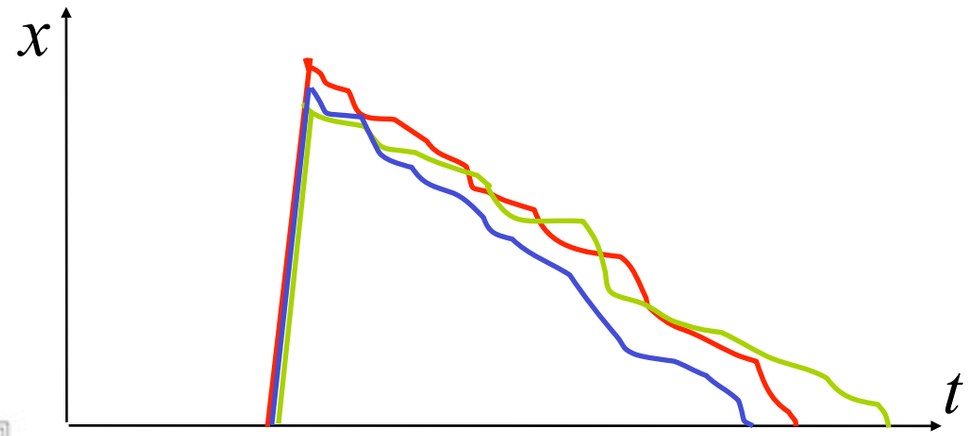
Mather, Bennett, Hasty, Tsimring, *PRL* 2009

$$\varnothing \xrightarrow{\alpha\left[1+x(t-\tau)/C_0\right]^{-2}} x$$

$$x \xrightarrow{\delta} \varnothing$$

For $C_0 \to 0$ these two reactions do not overlap in time

Period variability:



Two sources of variability (both Poissonian):

- fluctuations of x_{max}

$$v_x = \left\langle x_{\max} \right\rangle = \alpha\tau$$

- fluctuations of decay time

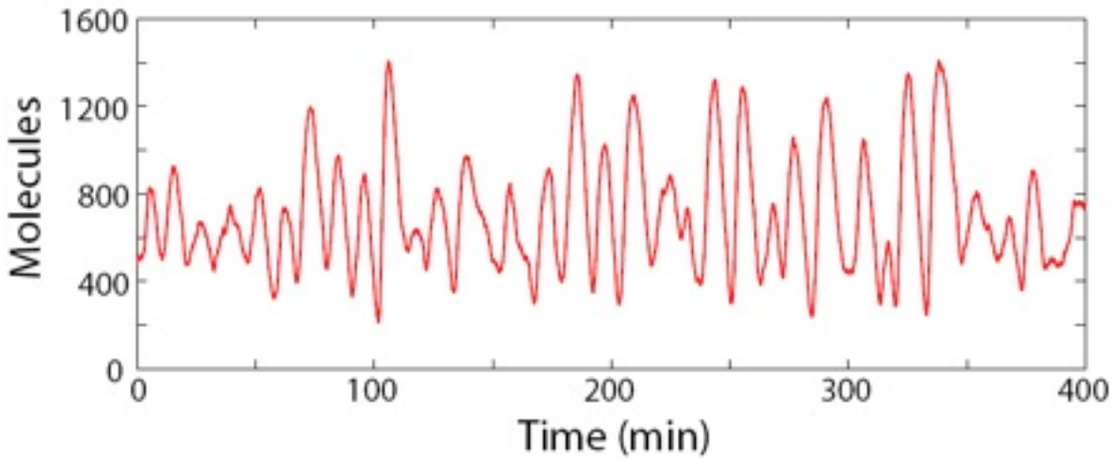
$$v_d = \left\langle T_d \right\rangle = \left\langle x_{\max} \right\rangle / \delta = \alpha\tau / \delta$$

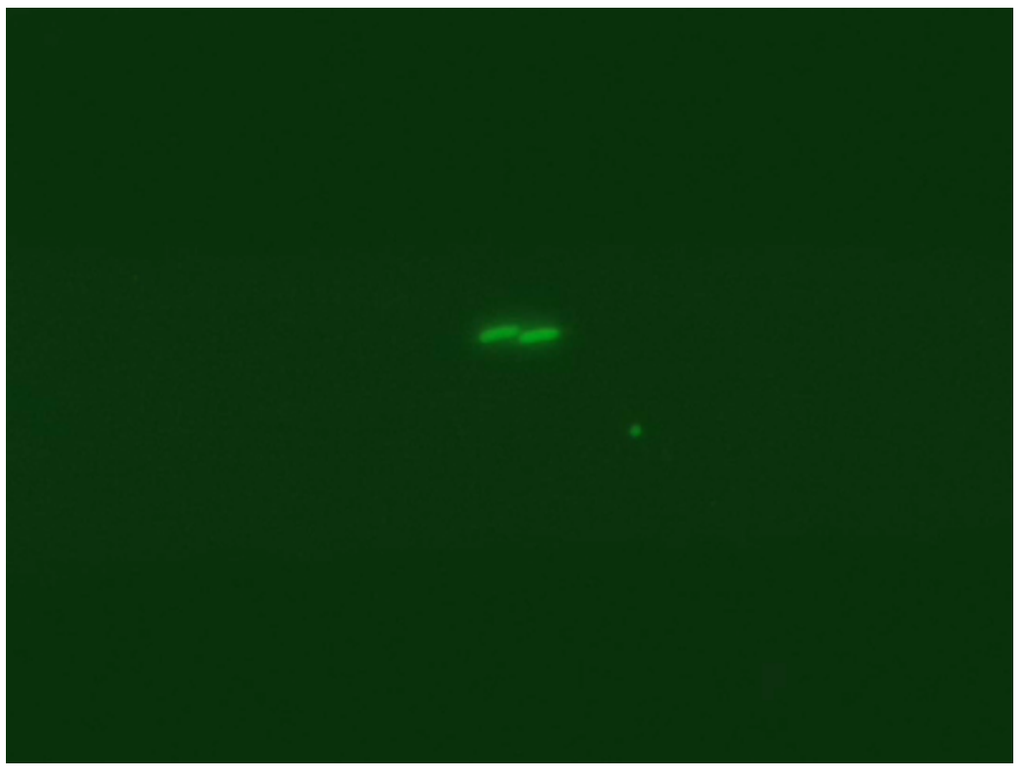
Period variance:

$$v = v_d + v_x / \delta = 2\alpha\tau / \delta$$

Negative feedback only system

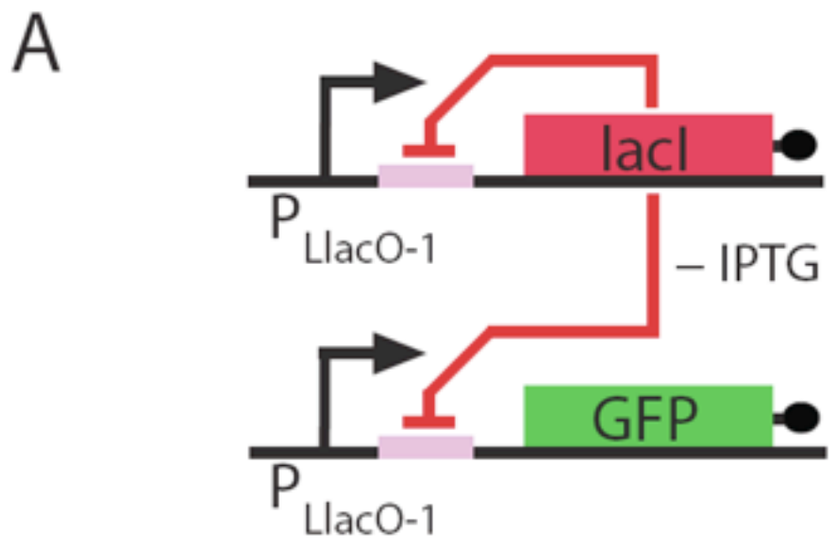
Full model (with constitutive activator):

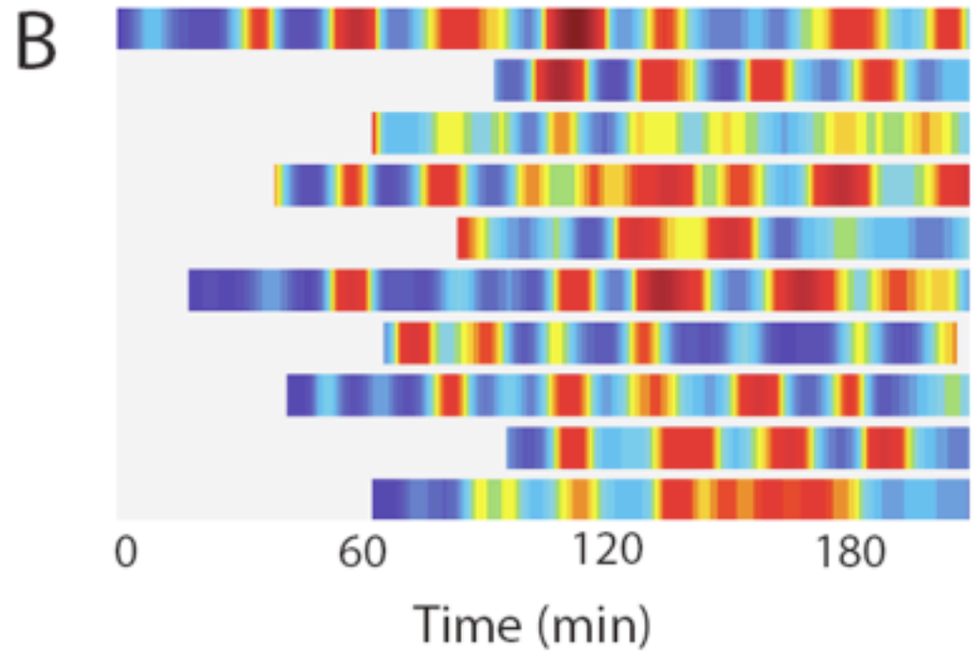




Also oscillates, but not as strong and robust

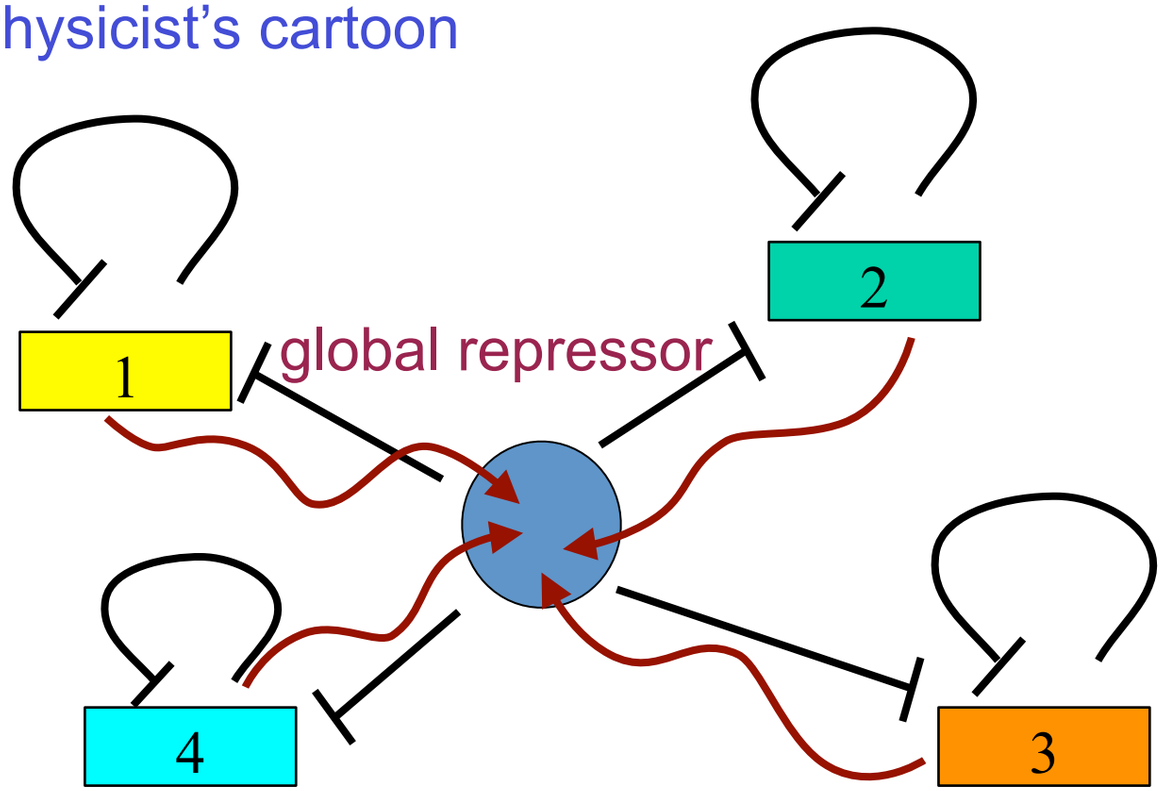
Experiment:





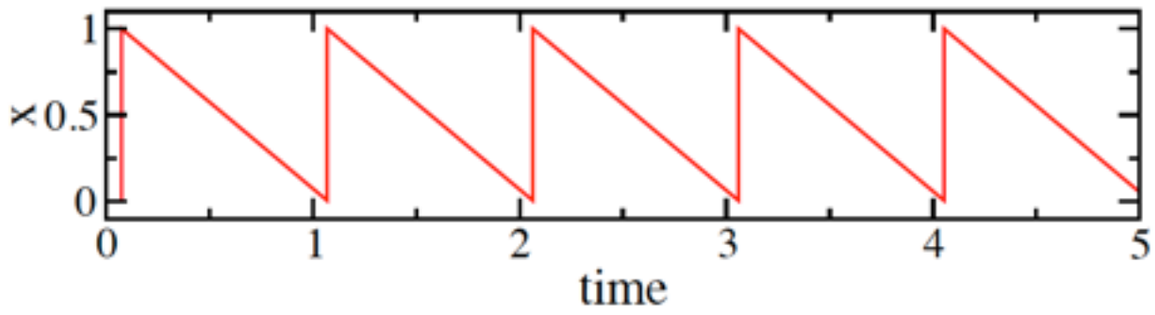
Synchronization of degrade-and-fire oscillators

Physicist's cartoon



single DF oscillator (discontinuous model)

- $\dot{x} = -1$
- when $x = \eta$, x reset to 1

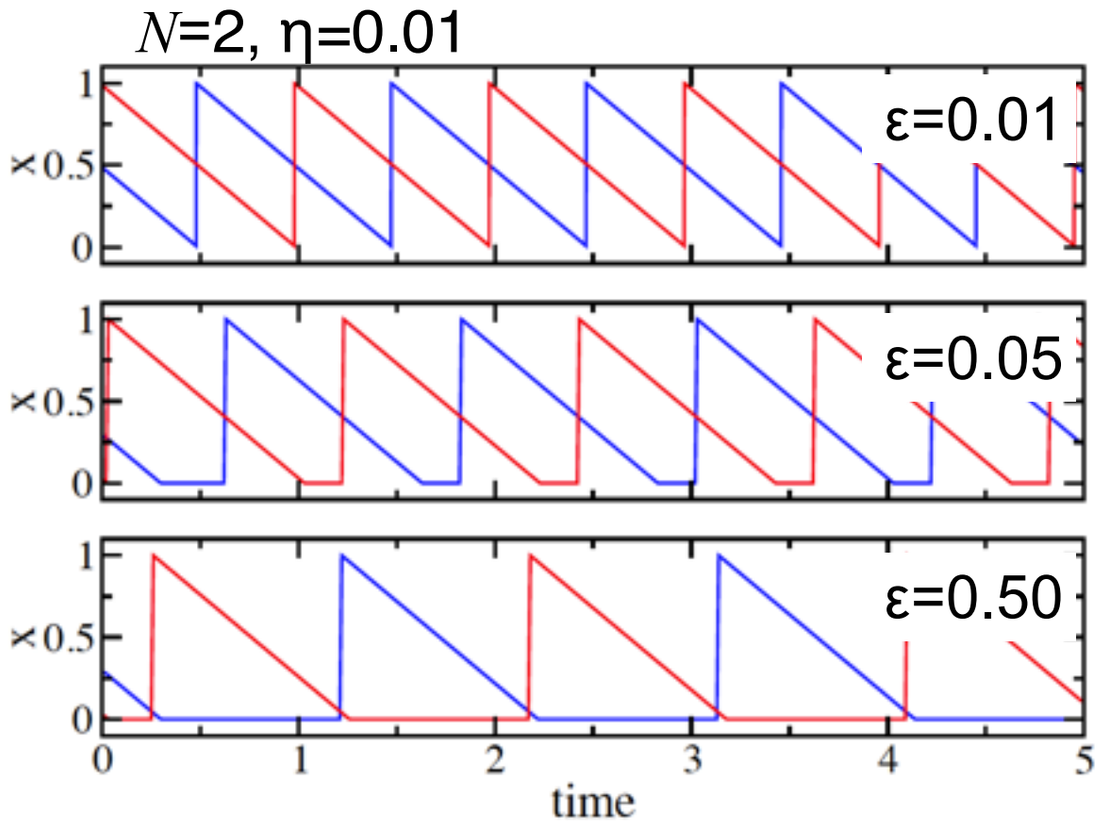


two DF oscillators:

- $\dot{x}_i = -\Theta(x_i), i = 1, 2$
- when $\xi_i \equiv \epsilon x_{3-i}/2 + (1 - \epsilon/2)x_i = \eta$, x_i reset to 1

Lemma: for any $\epsilon > 0$ and η a unique stable periodic orbit exists.

anti-phase synchronization, no collapse!



with Bastien Fernandez

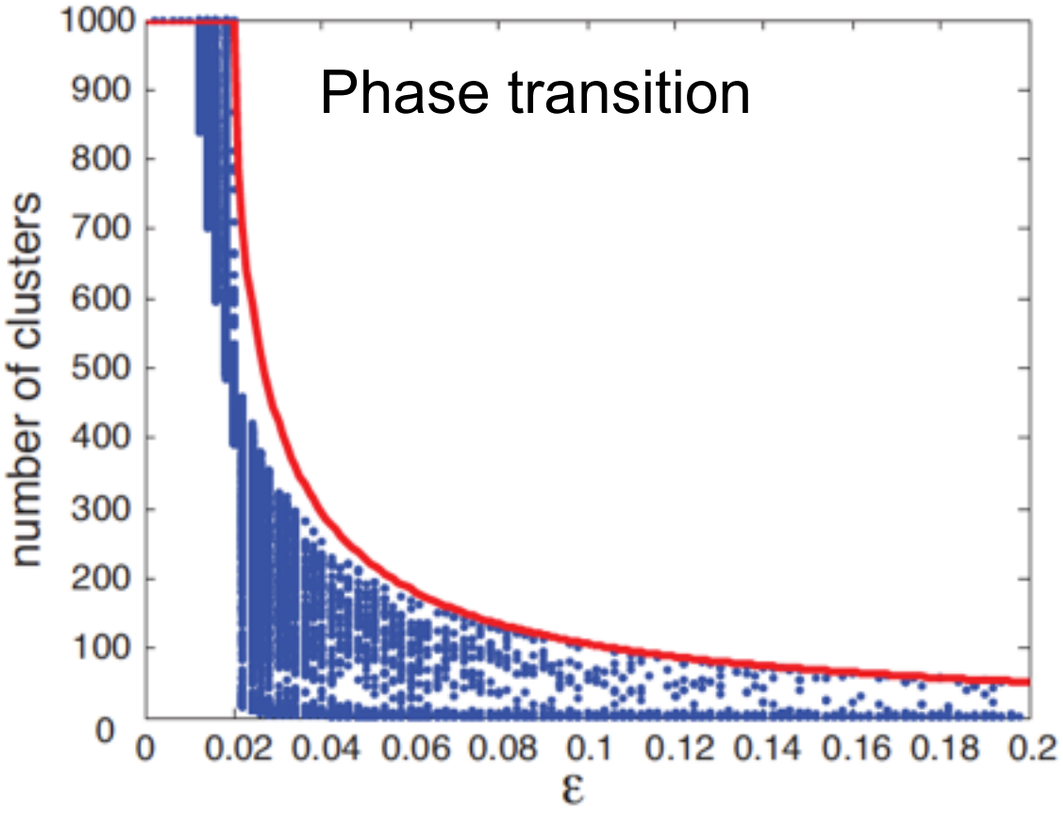
Co-repressive coupling of many DF oscillators

many oscillators:

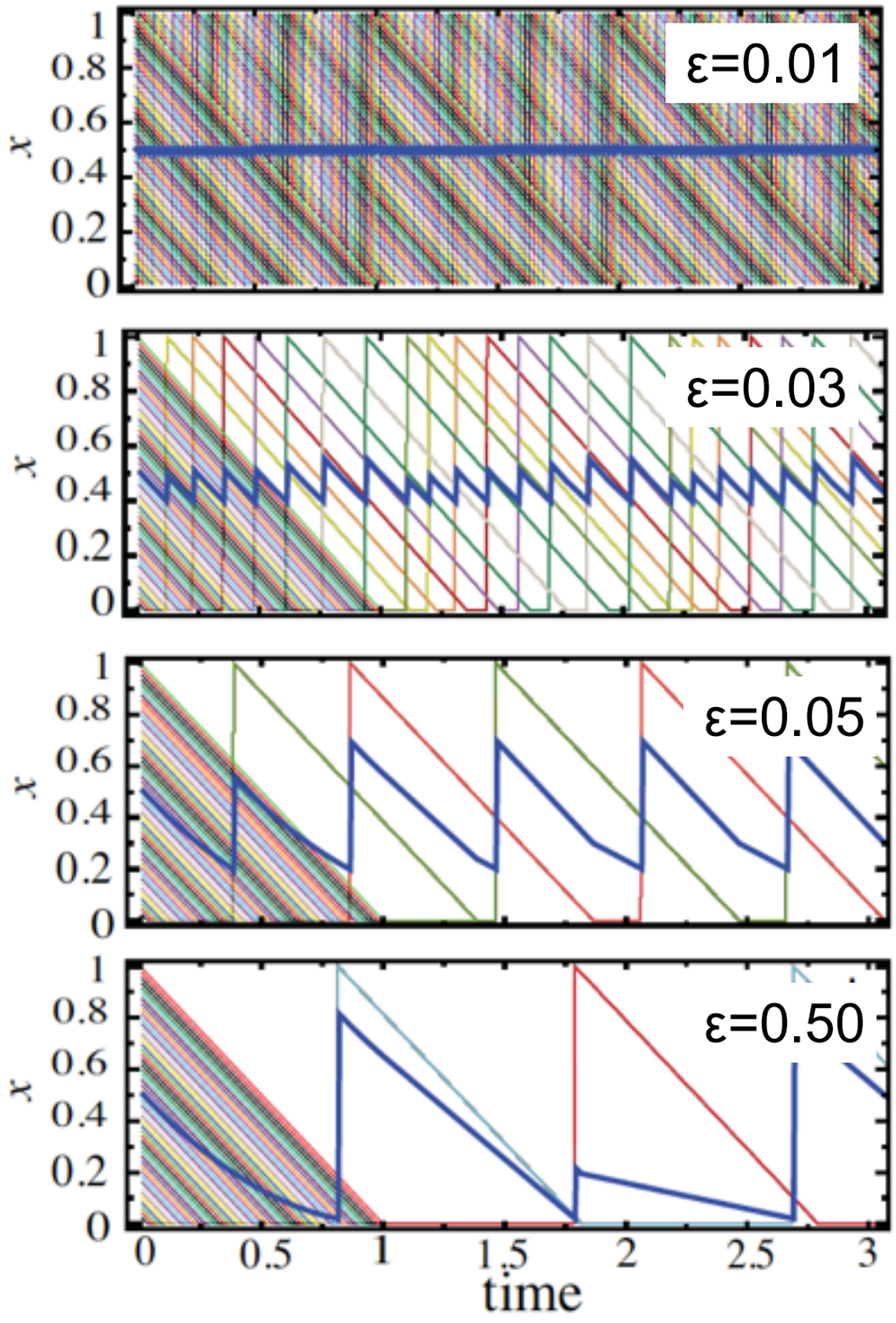
- $\dot{x}_i = -\gamma_i \Theta(x_i), i = 1, ..., N$
- mean field $X = N^{-1} \sum_i x_i$
- when $\xi_i \equiv \epsilon X + (1-\epsilon) x_i = \eta$, x_i reset to 1

Exact upper bound for the number of clusters:

$$K_u = N - \left\lfloor \tfrac{1}{2} + \sqrt{\tfrac{1}{4} + N(N-1)\left(1 - \tfrac{2N\eta}{\epsilon(N-2)}\right)} \right\rfloor$$

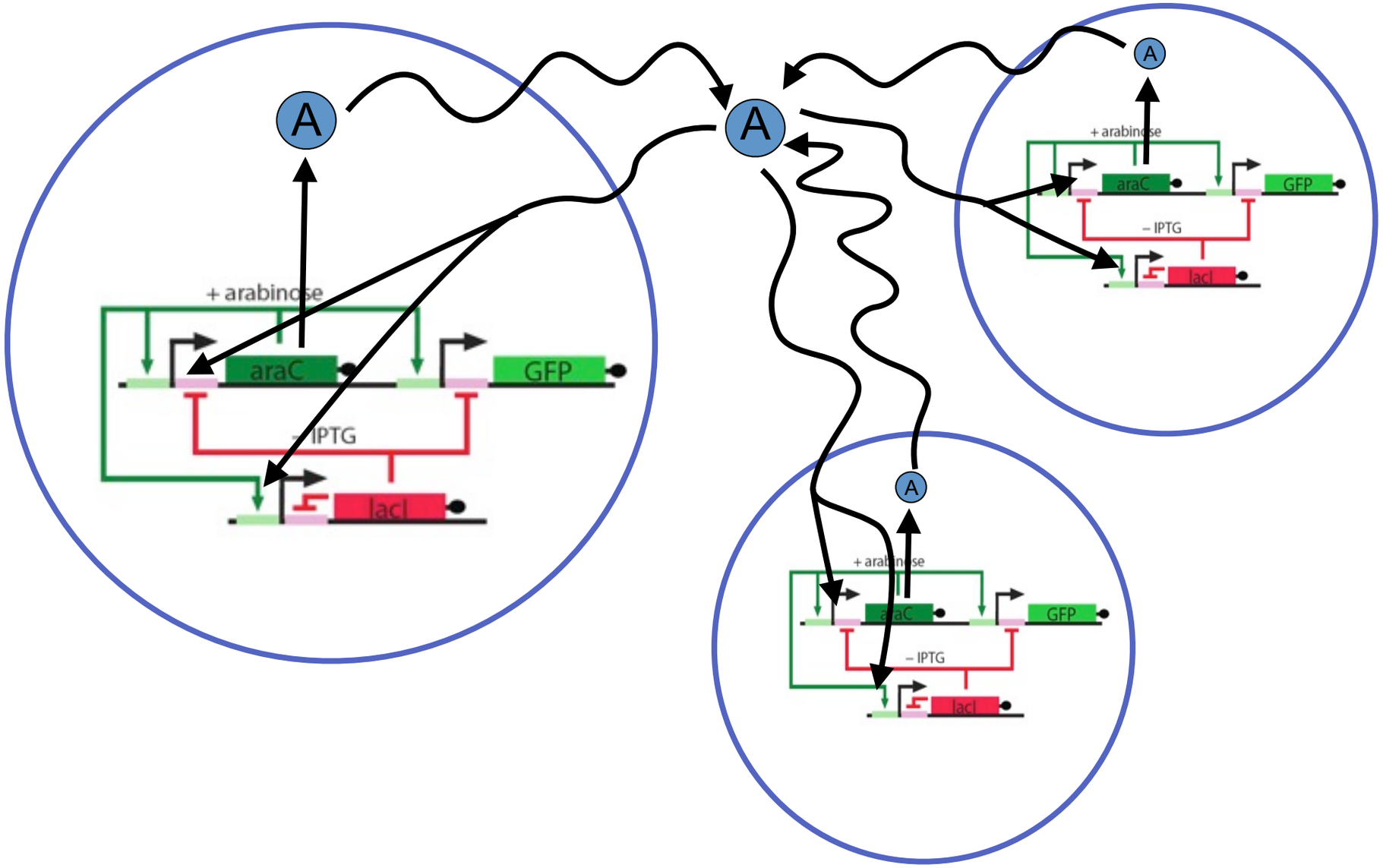


N=50, η=0.01



with Bastien Fernandez

Quorum-sensing synchronization of degrade-and-fire oscillators



Quorum-sensing molecules freely diffuse in and out of cells

Quorum-sensing synchronization of degrade-and-fire oscillators

Model equations (NF only):

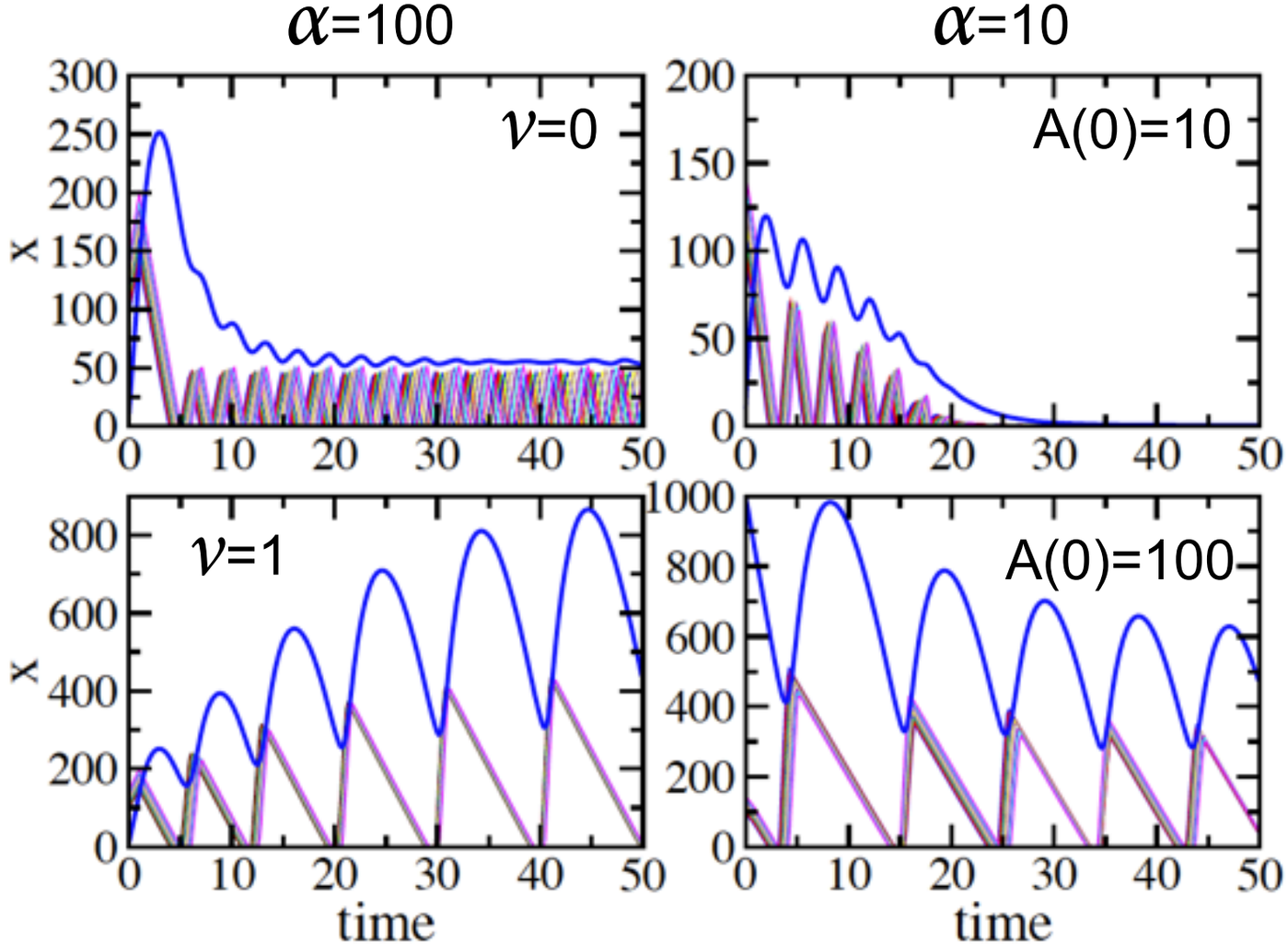
$$\frac{dx_i}{dt} = \frac{\alpha_i + \nu A_{\tau_2}}{(1 + x_{i,\tau_1}/C_0)^2} - \frac{\gamma_i x_i}{K_i + x_i}, \; i = 1, ..., N$$

$$\frac{dA}{dt} = \frac{1}{N}\sum_i x_i - \beta A$$

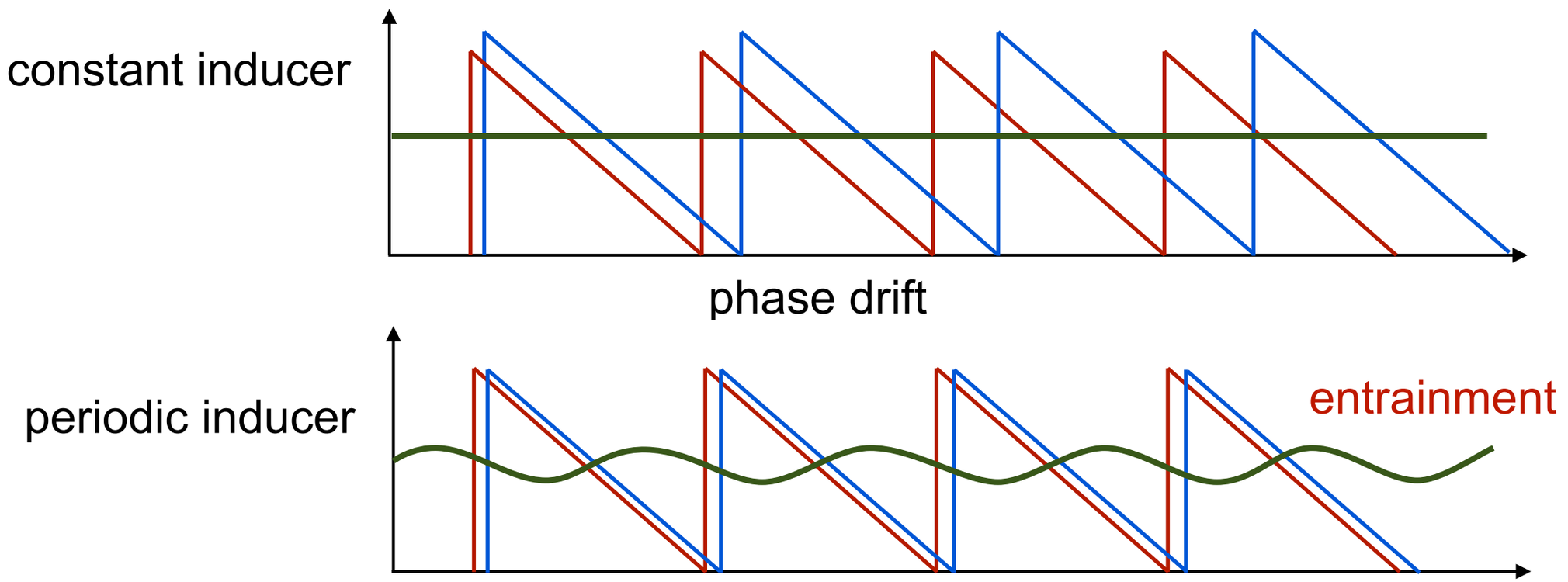
Examples:

$\tau_1 = 1; \tau_2 = 2; \beta = 0.3;$
$\gamma_i = 50; C_0 = 2; K_i = 1$

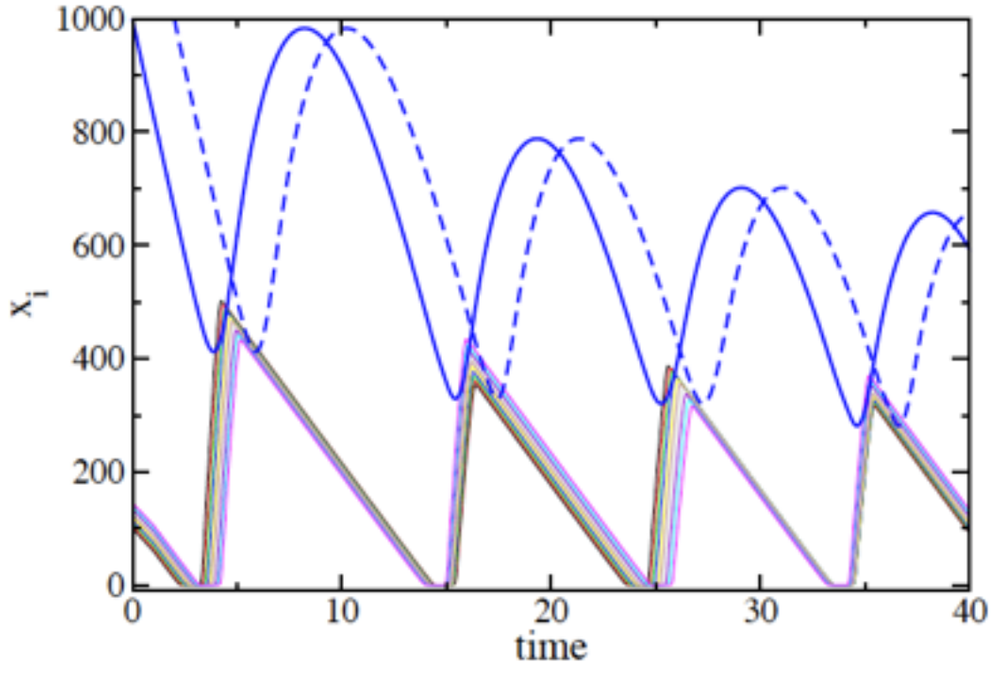
10% variability

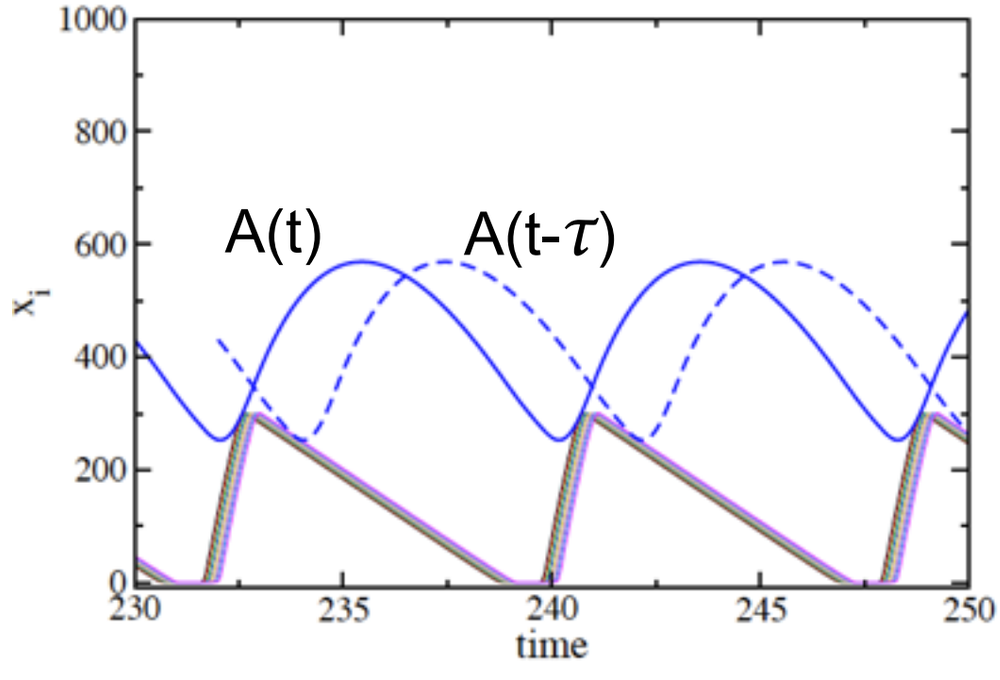


Mechanism of synchronization



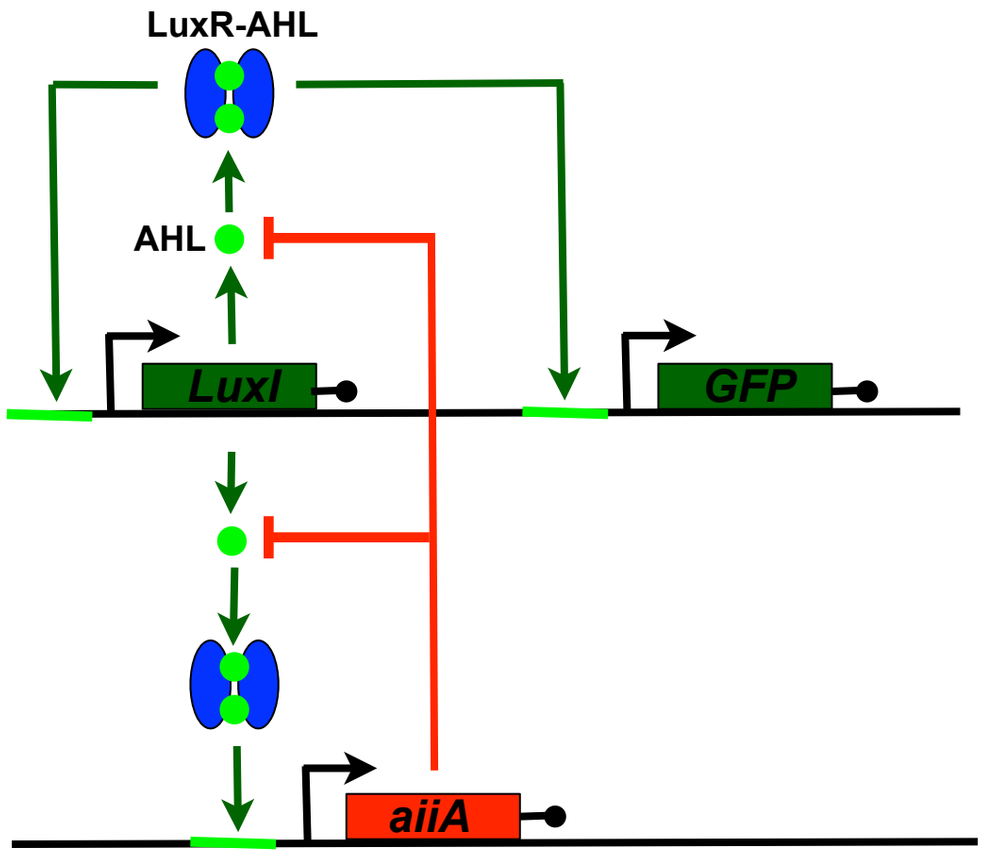
burst localization on the falling part of the *delayed* AHL profile stabilizes phase drift: self-entrainment





Synchronized quorum of genetic clocks

Core oscillator is similar (activated repression), but different components



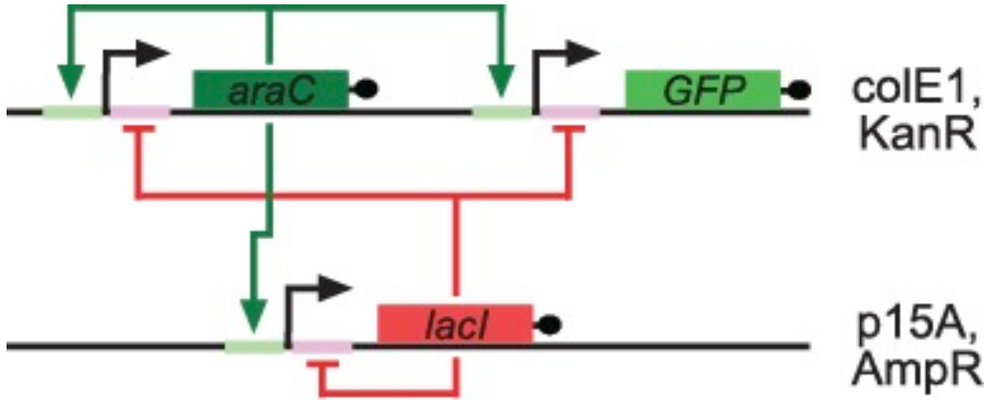
LuxI - synthase enzyme which chops fatty acid molecules in the cell to make AHL (from *V. fischeri*)

AHL - binds to luxR and the complex activates the *luxI* promoter

AiiA - enzyme which degrades AHL (from *B. thuringiensis*)

Coupling: AHL freely diffuses in and out of cell

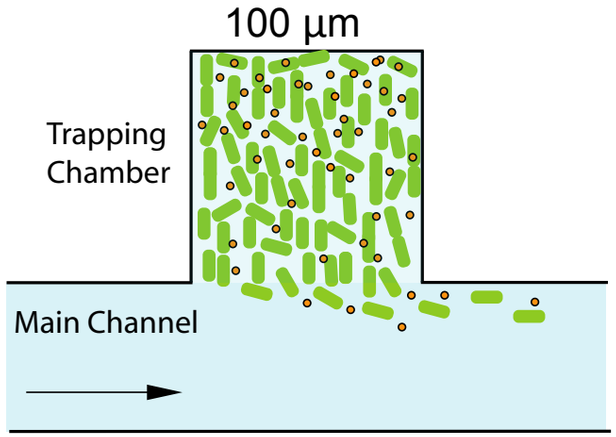
Compare with:



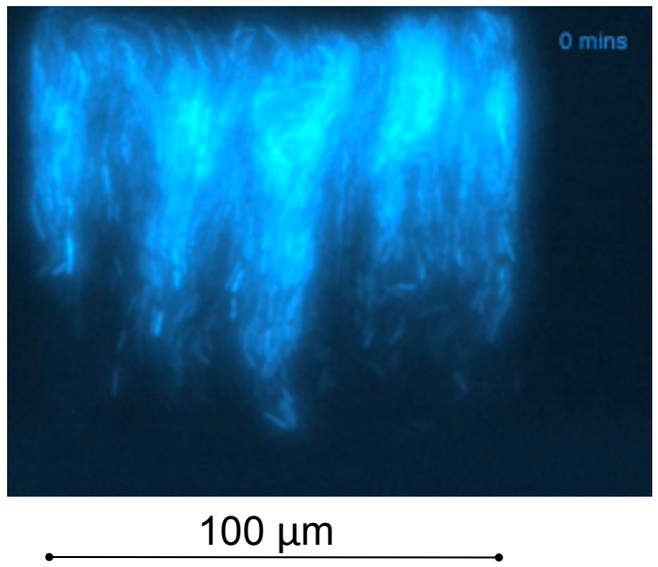
T. Danino *et al.*, *Nature* 2010

Microcolony in a side trap

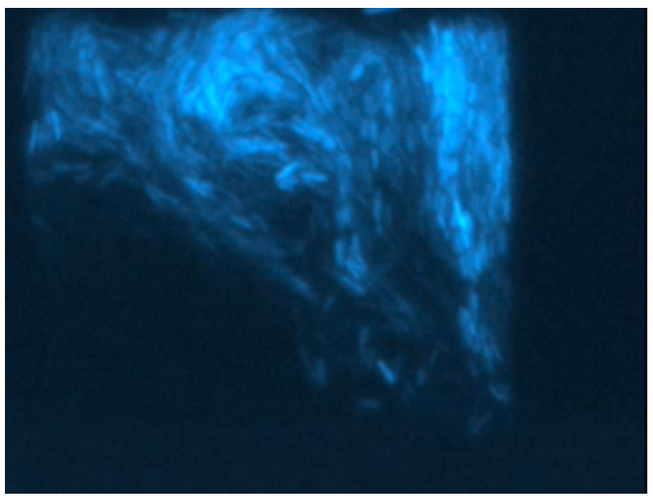
- Exponentially growing population for at least 4 days
- Flow rate modulates effective degradation of AHL

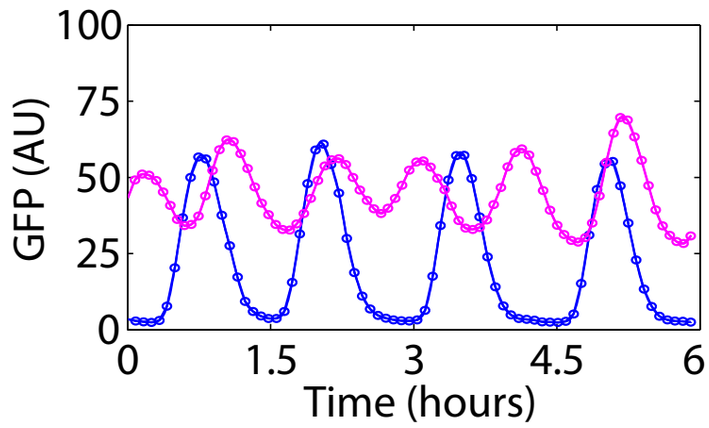


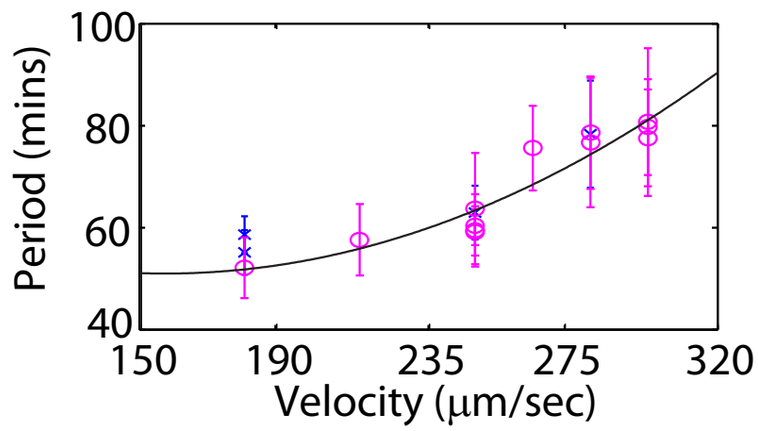
Low flow rate (~240 µm/min)



High flow rate (~280 µm/min)

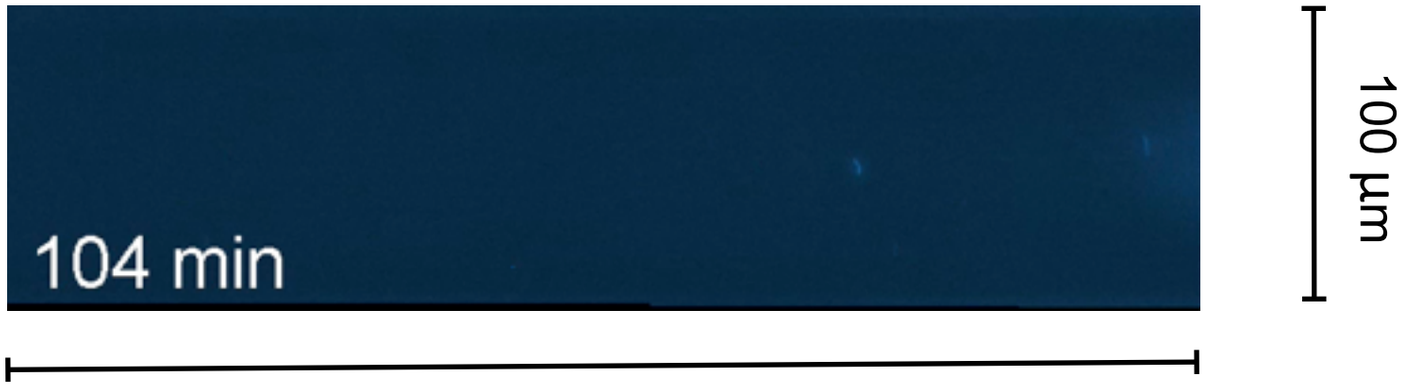


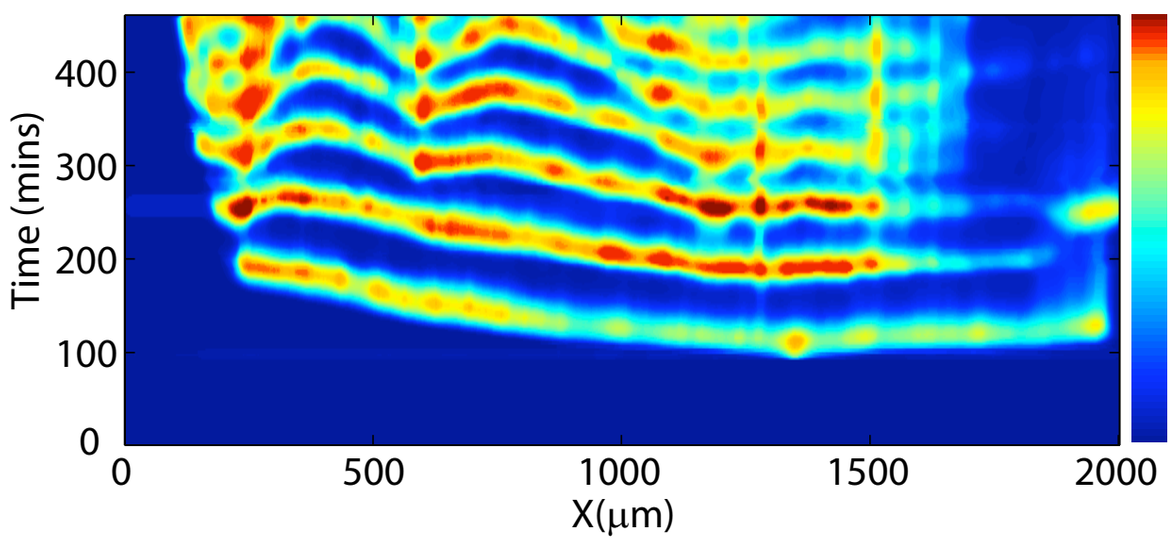




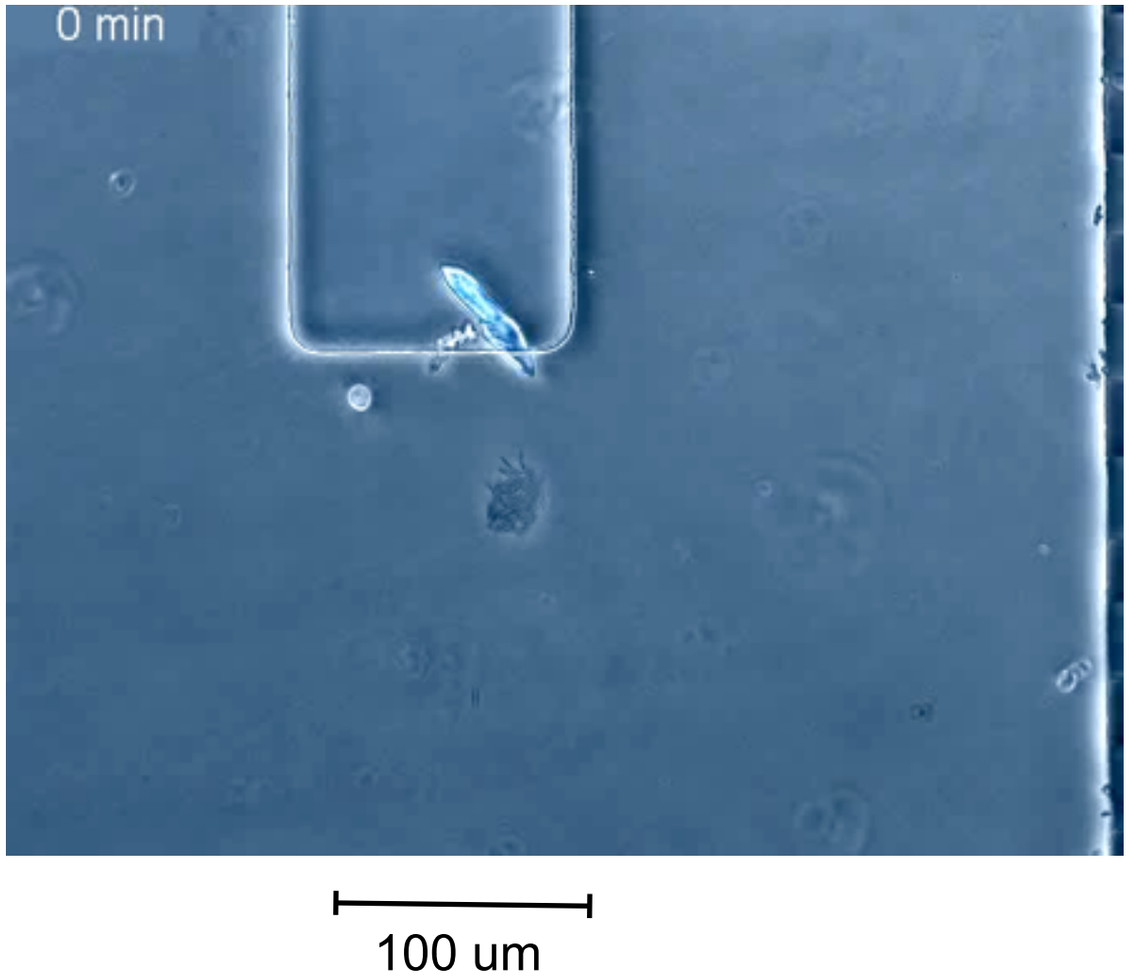
Traveling Waves of AHL

Extended 2D monolayer

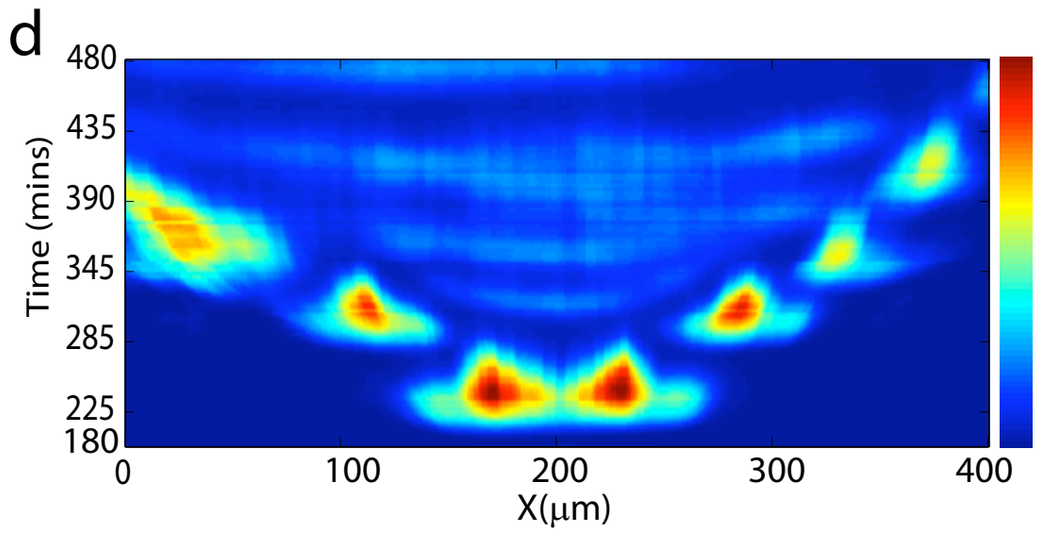




Growing 3D colony



Waves propagate at 8-12 μm/min



Detailed delayed feedback model

$$\frac{\partial A}{\partial t} = C_A[1-(d/d_0)^4]\,P(\alpha,\tau) - \frac{\gamma_A A}{1+f(A+I)}$$

$$\frac{\partial I}{\partial t} = C_I[1-(d/d_0)^4]\,P(\alpha,\tau) - \frac{\gamma_I I}{1+f(A+I)}$$

$$\frac{\partial H_i}{\partial t} = \frac{bI}{1+kI} - \frac{\gamma_H A H_i}{1+gA} + D(H_e - H_i)$$

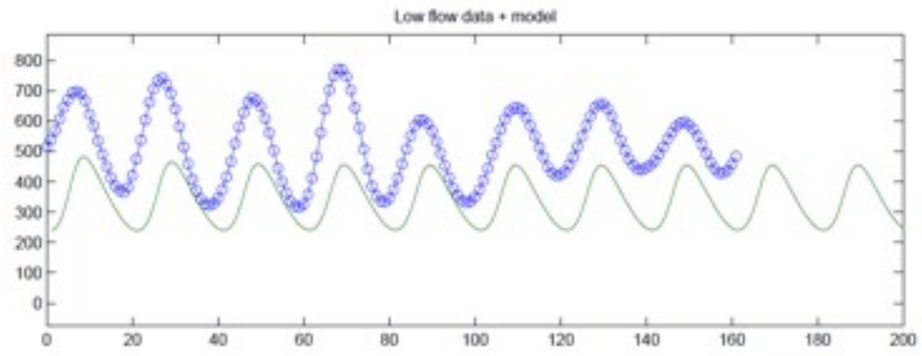
$$\frac{\partial H_e}{\partial t} = -\frac{d}{1-d}D(H_e - H_i) - \mu H_e + D_1\frac{\partial^2 H_e}{\partial x^2}$$

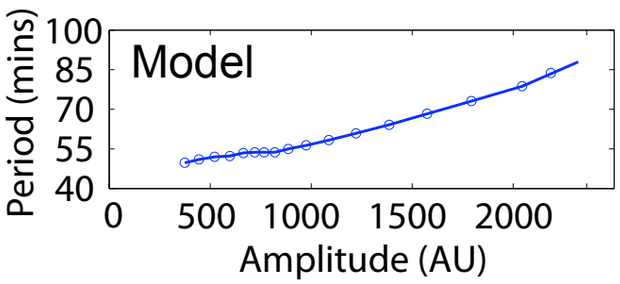
$$P(\alpha,\tau) = \frac{\delta + \alpha H_\tau^2}{1+k_1 H_\tau^2}$$

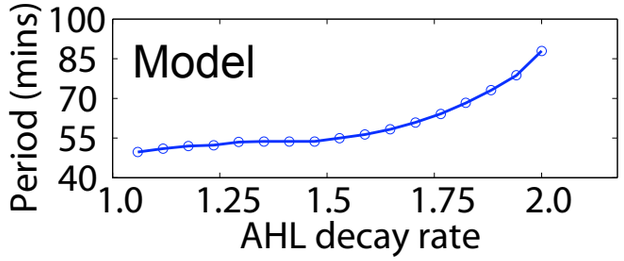
delayed synthesis

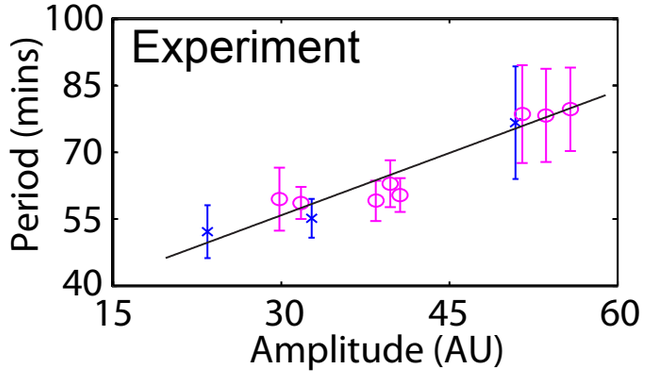
spatial diffusive coupling

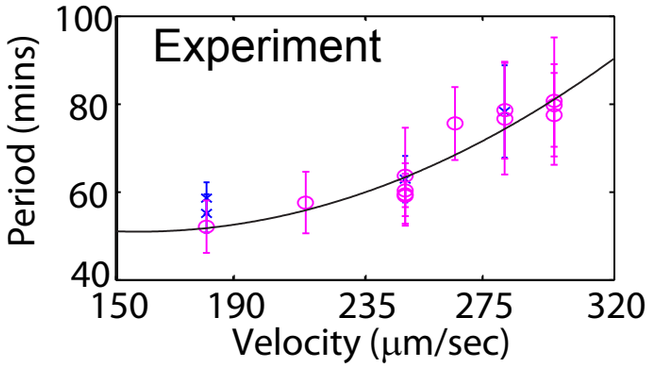
- A=aiiA, I=LuxI,H_i=internal AHL, H_e=external AHL
- luxR is at a constant level
- Production of aiiA/luxI is delayed
- Diffusion is slow compared to external flow rate



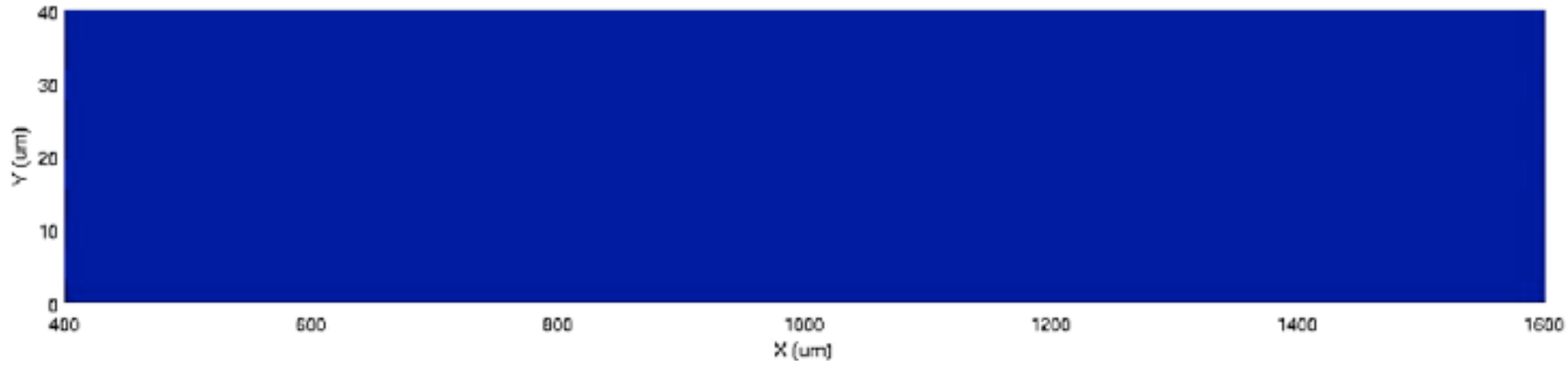




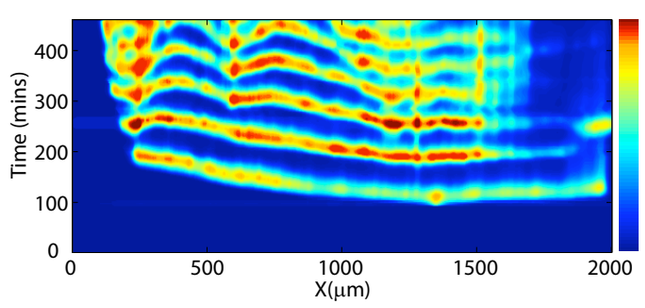


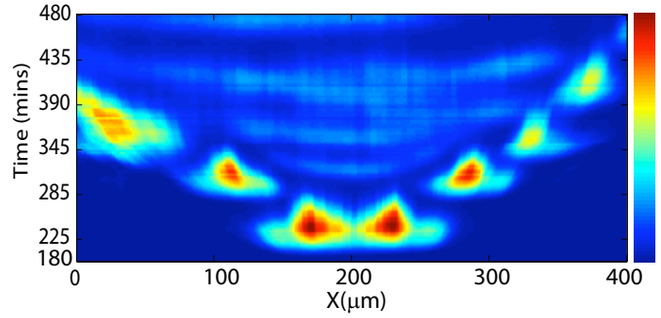


Space-time model simulations

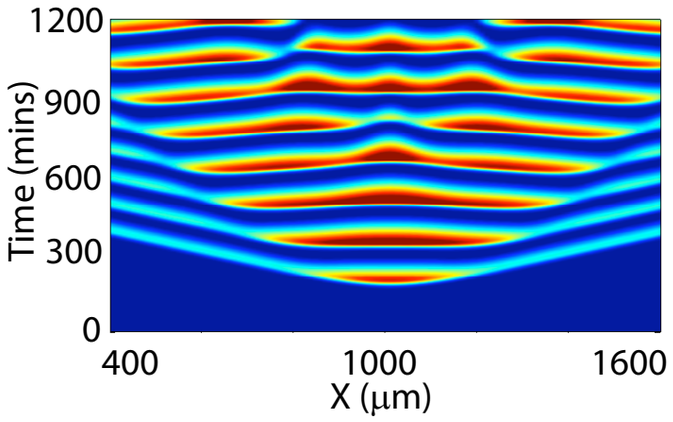


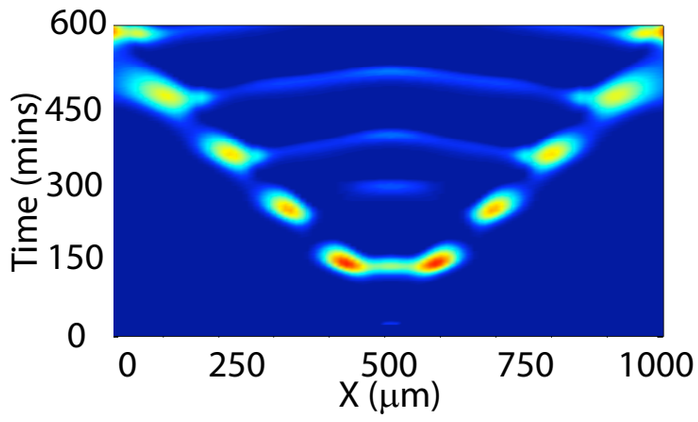
Experiments





Simulations





Conclusions

- Genetic circuits are prone to *oscillations*
- Biologically relevant oscillatory motif: Delayed auto-repression. Two-gene oscillator (activated auto-repression) designed and implemented in *E.coli*
- Robust (small)-delay-induced oscillations: degrade-and-fire mechanism
- Oscillators in different cells can be entrained and synchronized by external chemical signal (inducer)
- Synchronized oscillations in a dense multi-cell populations: similar design, different components
- Co-repressive mechanism explains synchronization in multi-trap experiments

- *D.A. Bratsun, D.N. Volfson, L.S. Tsimring, J. Hasty.* Delay-induced oscillations in gene regulation. PNAS, **102** (41), 14593-8(2005).
- *J. Stricker, S. Cookson, M. Bennett, W. Mather, L. S. Tsimring, J. Hasty.* A robust and tunable synthetic gene oscillator. Nature, **456**(7221):516-9 (2008).
- *W. Mather, M. R. Bennett, J. Hasty, L. S. Tsimring,* Delay-induced degrade-and-fire oscillations in small genetic circuits. Phys. Rev. Lett., **102**, 068105 (2009).
- *T. Danino, O. Mondrgon-Palomino, L. S. Tsimring, J. Hasty*, A synchronized quorum of genetic clocks, *Nature*, **463**, doi10/1038/nature08753 (2010).

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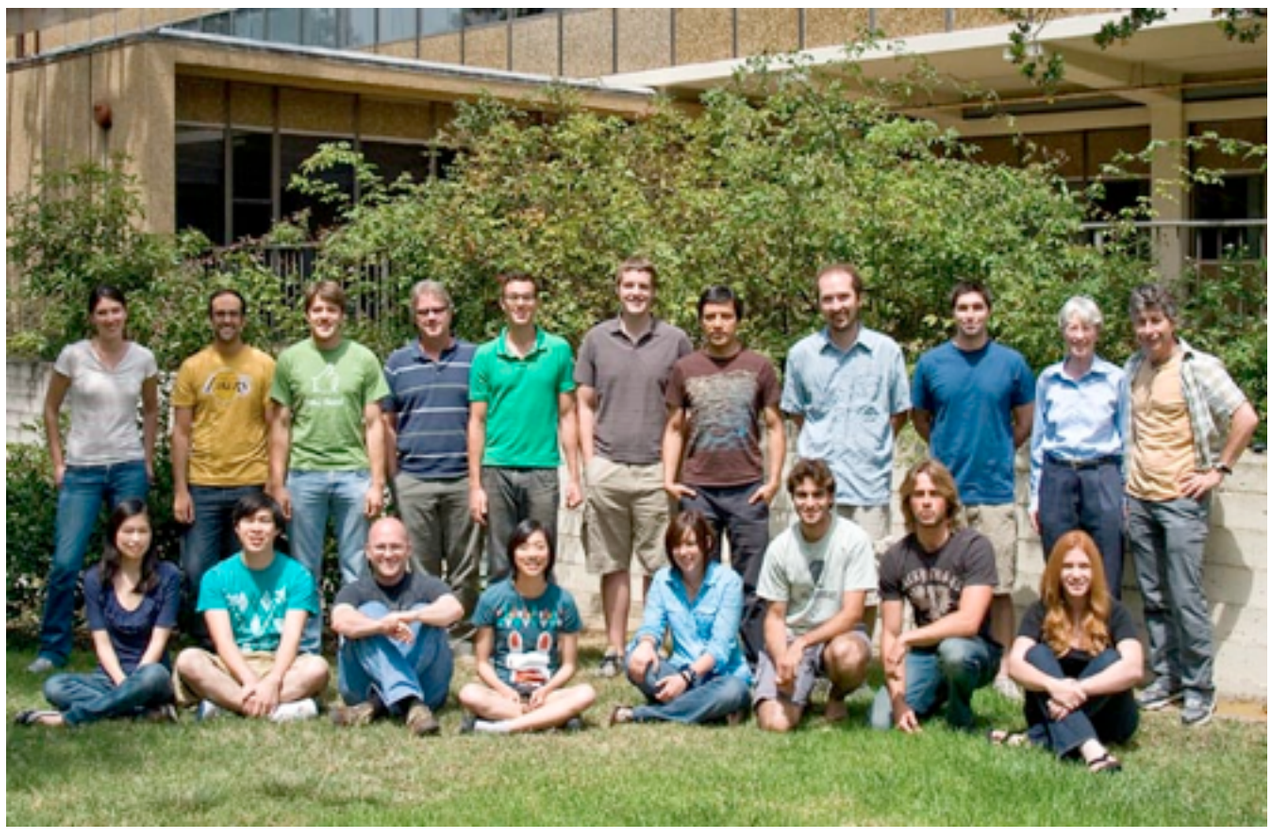
Modeling

Dmitri Bratsun
Dmitri Volfson
Matt Bennett
William Mather
Phillip Samayoa

Bastien Fernandez (Marseille)



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