Stochastic dynamics of small gene regulation networks

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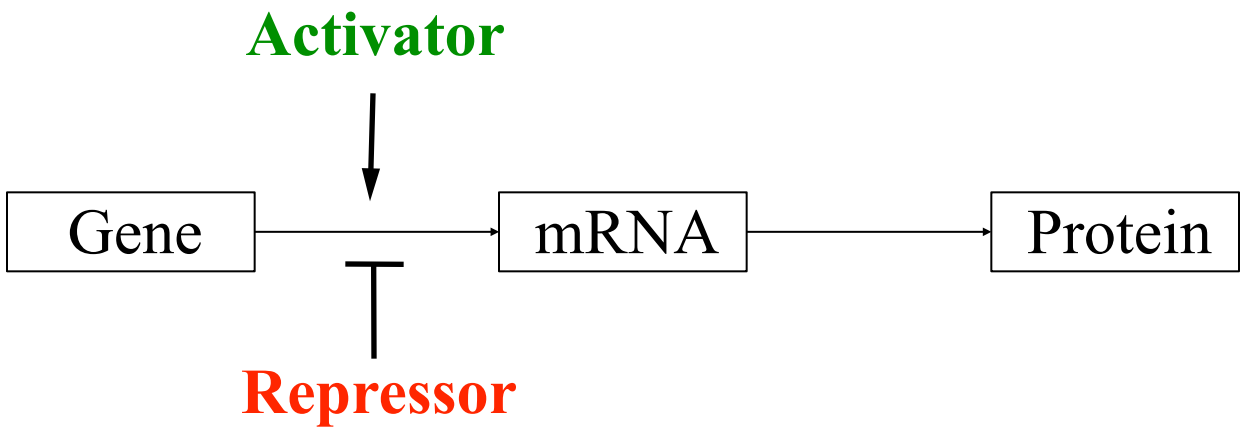
Nizhni Novgorod, June, 2011

BCI

Central dogma

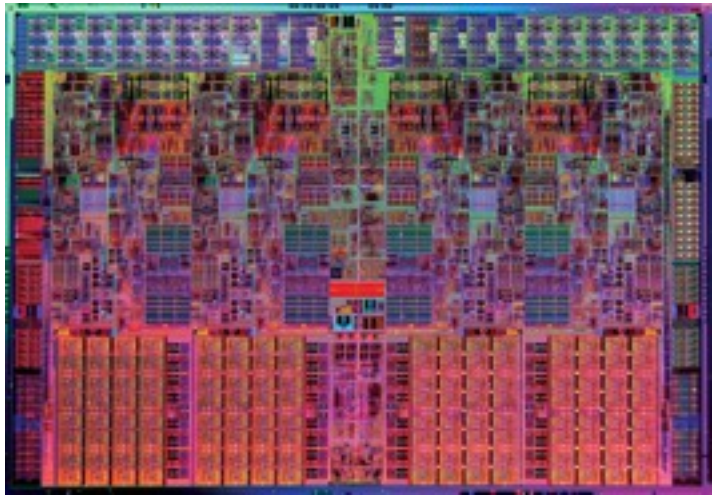


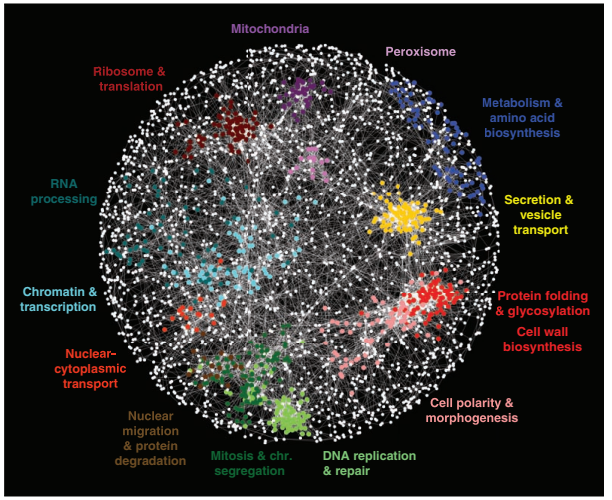
All cells have the same genes…
…So why cell differ from each other?

Transcriptional Regulation

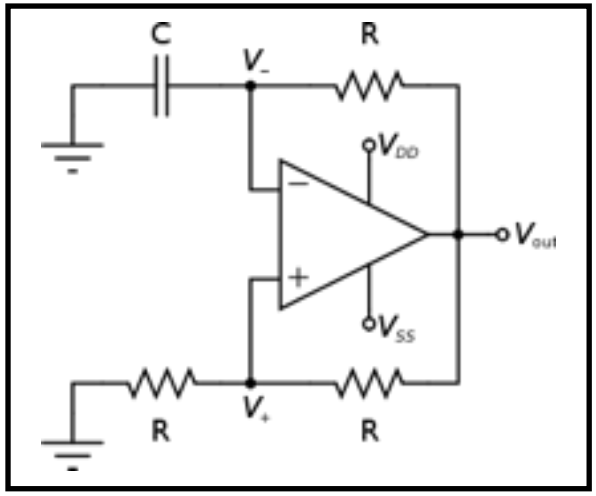
Activator – increases rate of production
Repressor – decreases rate of production

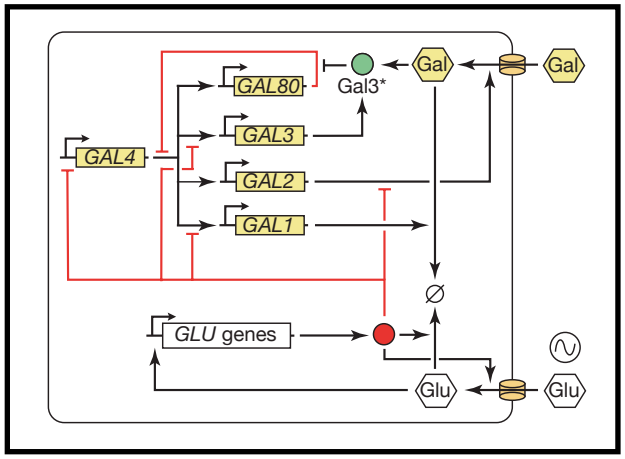
Systems Biology

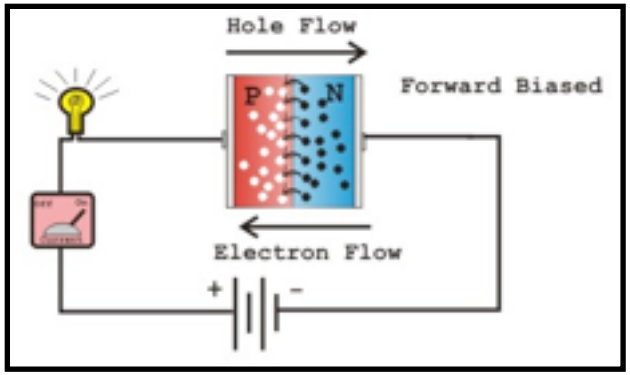


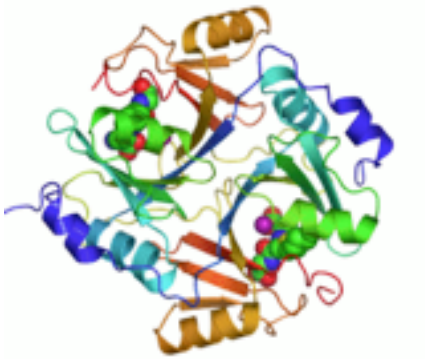


scale



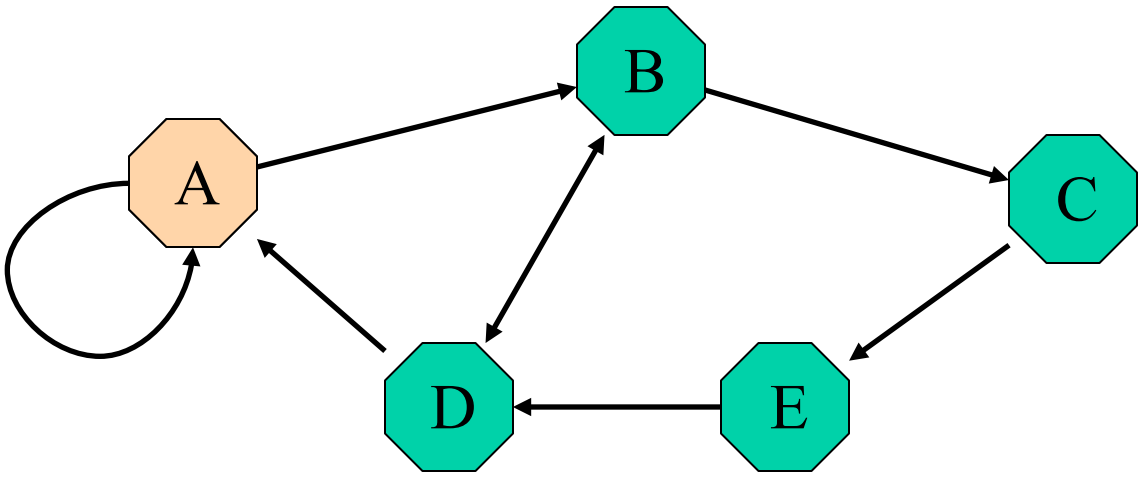




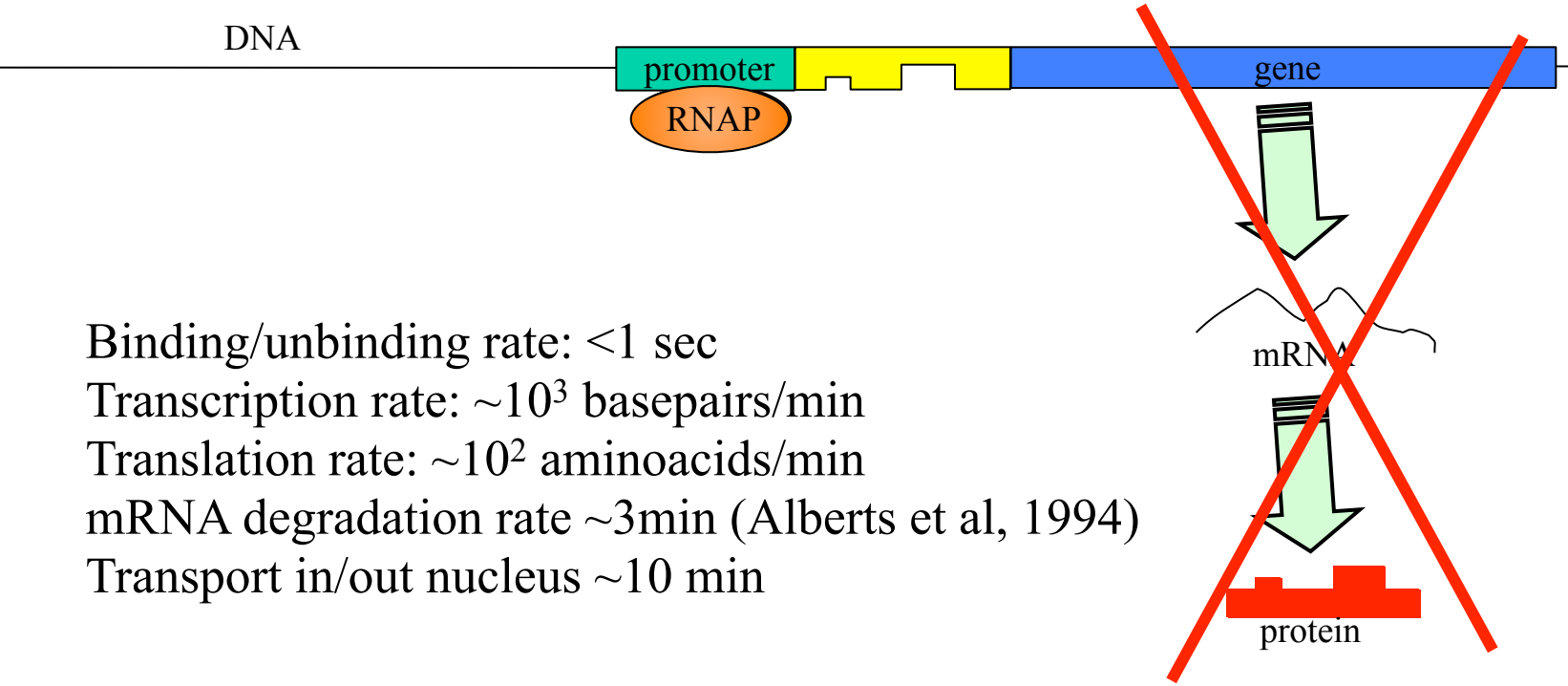


Gene regulation networks

- Proteins affect rates of production of other proteins (or themselves)
- This leads to formation of *networks* of interacting genes/proteins
- Large stochastic fluctuations
- Sub-networks are *non-Markovian*, even if the whole system is
- Different reaction channels operate at vastly different time scales and number densities



Auto-repressor: A cartoon



Binding/unbinding rate: <1 sec
Transcription rate: ~10^3 basepairs/min
Translation rate: ~10^2 aminoacids/min
mRNA degradation rate ~3min (Alberts et al, 1994)
Transport in/out nucleus ~10 min

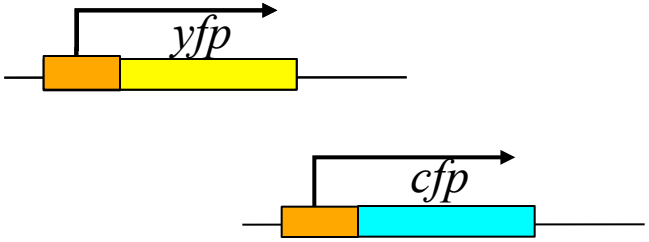
Vastly different time scales: need for reduced descriptions

Transients in gene regulation

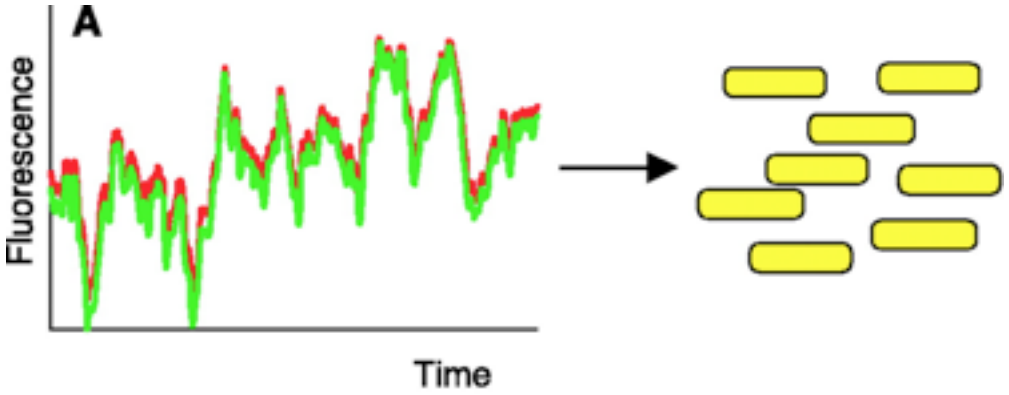
- Genetic circuits are never at a fixed point:
  - Intrinsic noise
  - Extrinsic noise
  - External signaling
  - Oscillations:

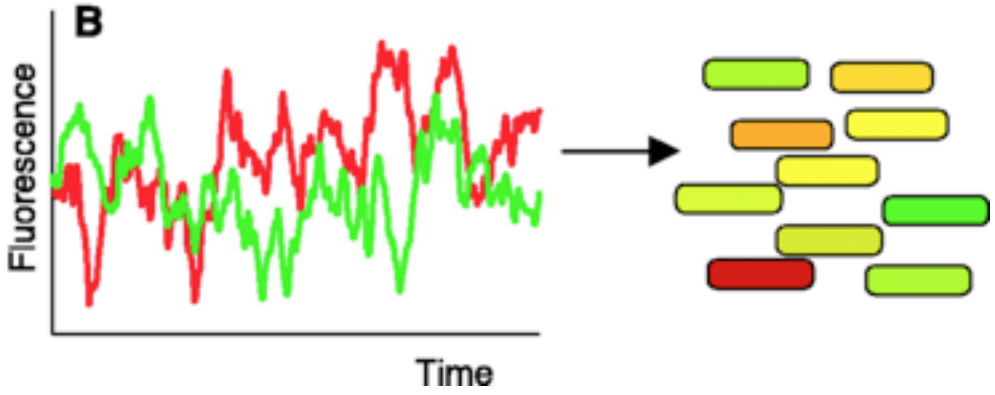
    circadian rhythms; ultradian rhythms; cell cycle

Intrinsic vs. Extrinsic noise?



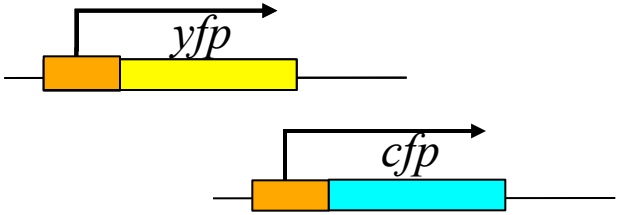
Identical promoters;
different reporter genes

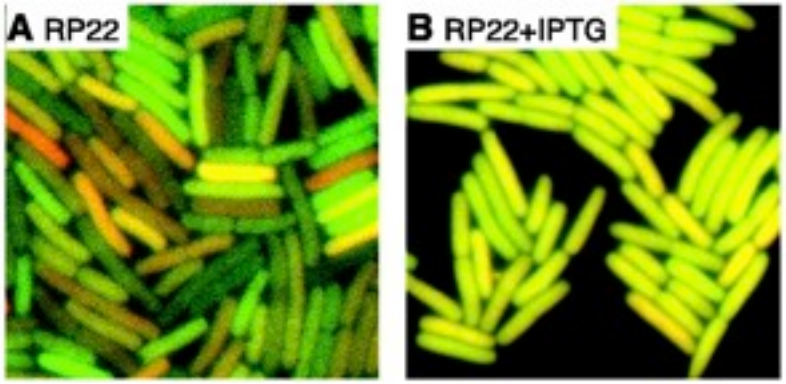




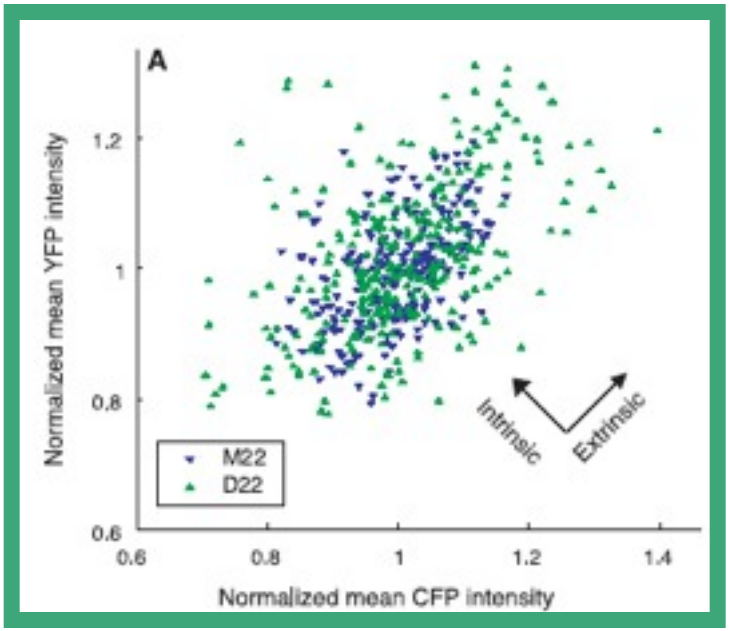
Elowitz et al, 2002

Intrinsic vs. Extrinsic noise?



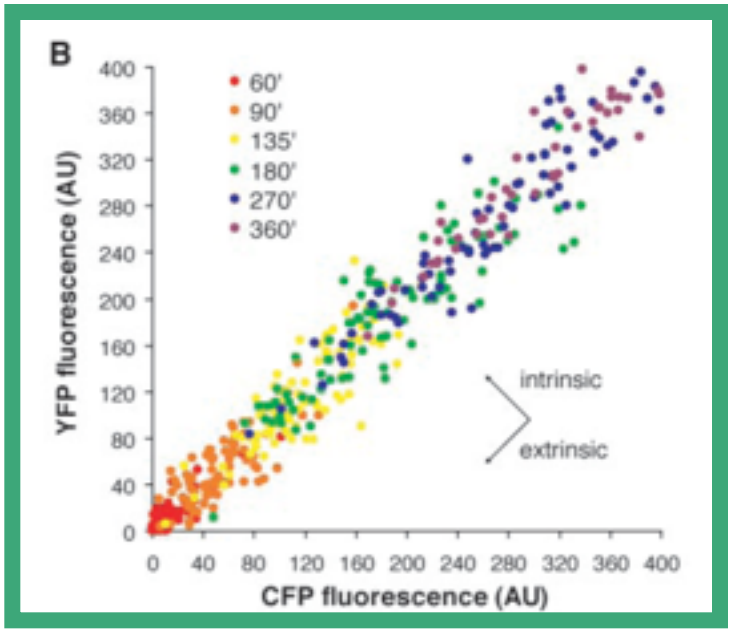


Bacteria



Elowitz et al, 2002

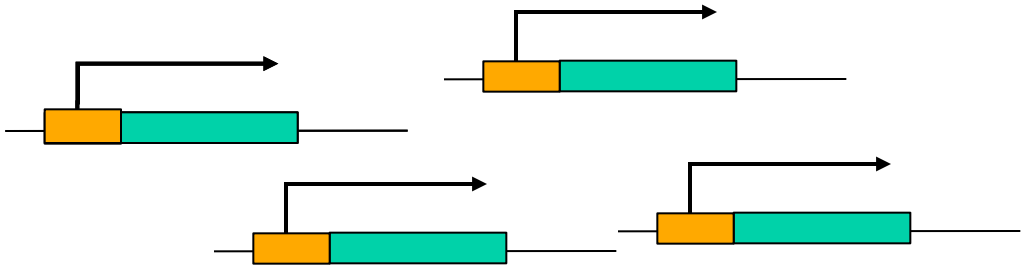
Yeast



Raser & O'Shea, 2004

Intrinsic noise is smaller in Yeast

Intrinsic vs. Extrinsic noise



Multiple identical promoters;
same reporter gene

Gene expression:

$$G_M = \sum_{i=1}^{M} \langle g_i \rangle; \quad V_M = \sum_{i=1}^{M} \left[\langle \tilde{g}_i^{\,2} \rangle + \sum_{j \neq i} \langle \tilde{g}_i \tilde{g}_j \rangle \right]$$

For identical promoter-gene pairs:

$$G_M = Mg; \quad V_M = Mv + M(M-1)c$$

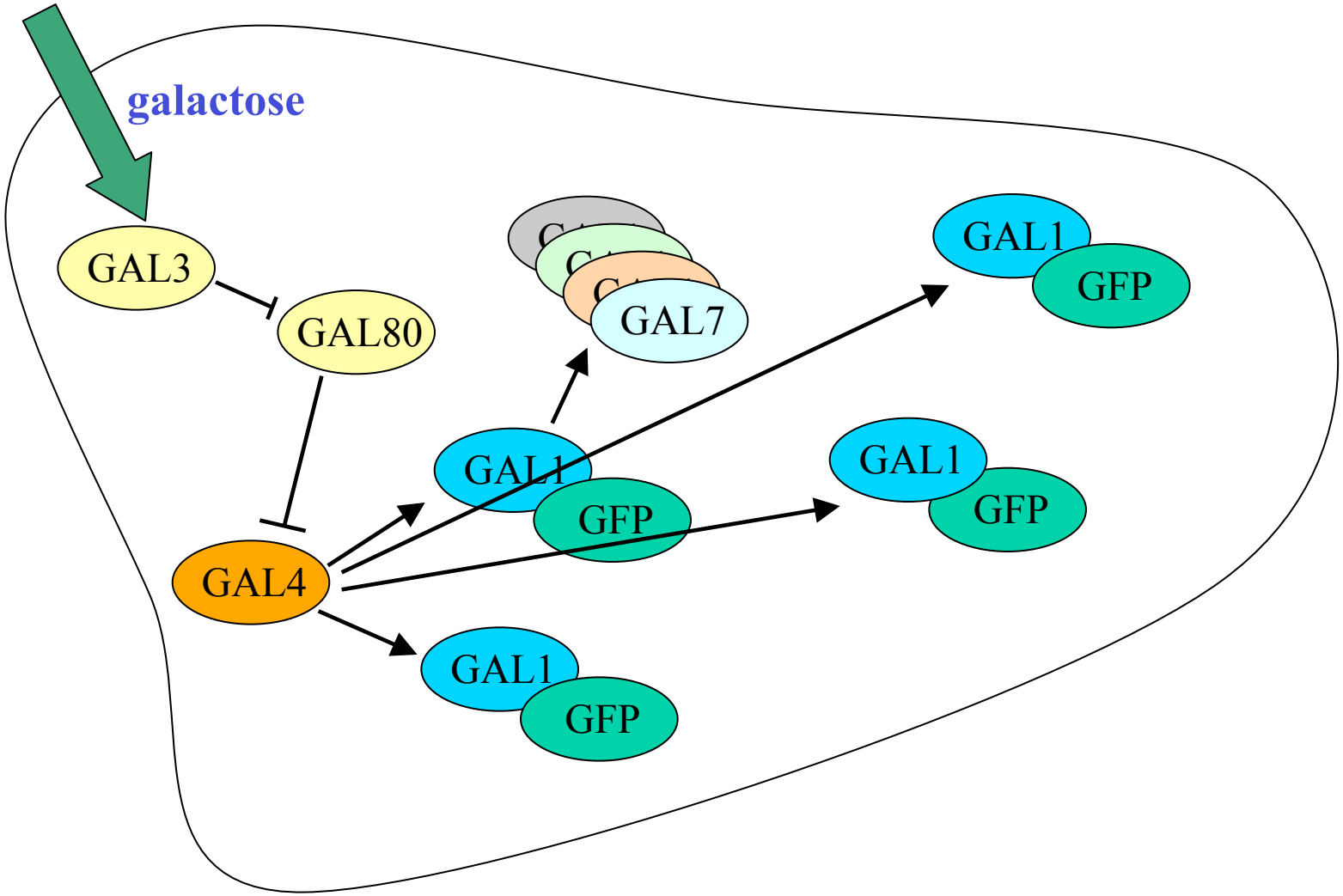
For intrinsic noise (c=0), $V_M = Mv; \quad CV \propto M^{-1/2}$

For correlated (extrinsic) noise (c=v), $V_M = M^2 v; \quad CV = const(M)$

Volfson et al., Nature, 2006

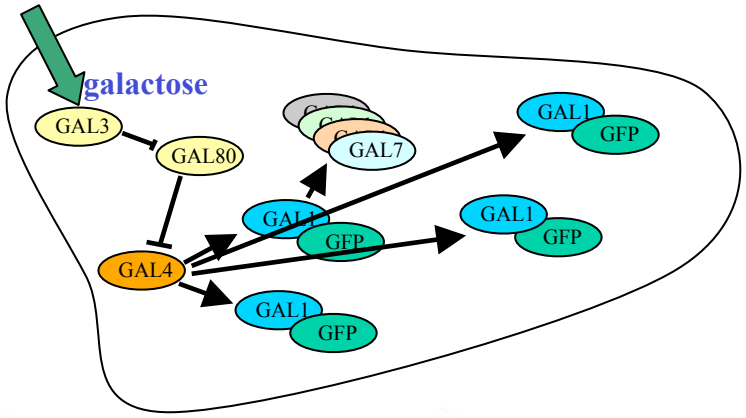
Intrinsic vs. Extrinsic noise

Saccaromyces cerevisiae (yeast); galactose utilization circuit



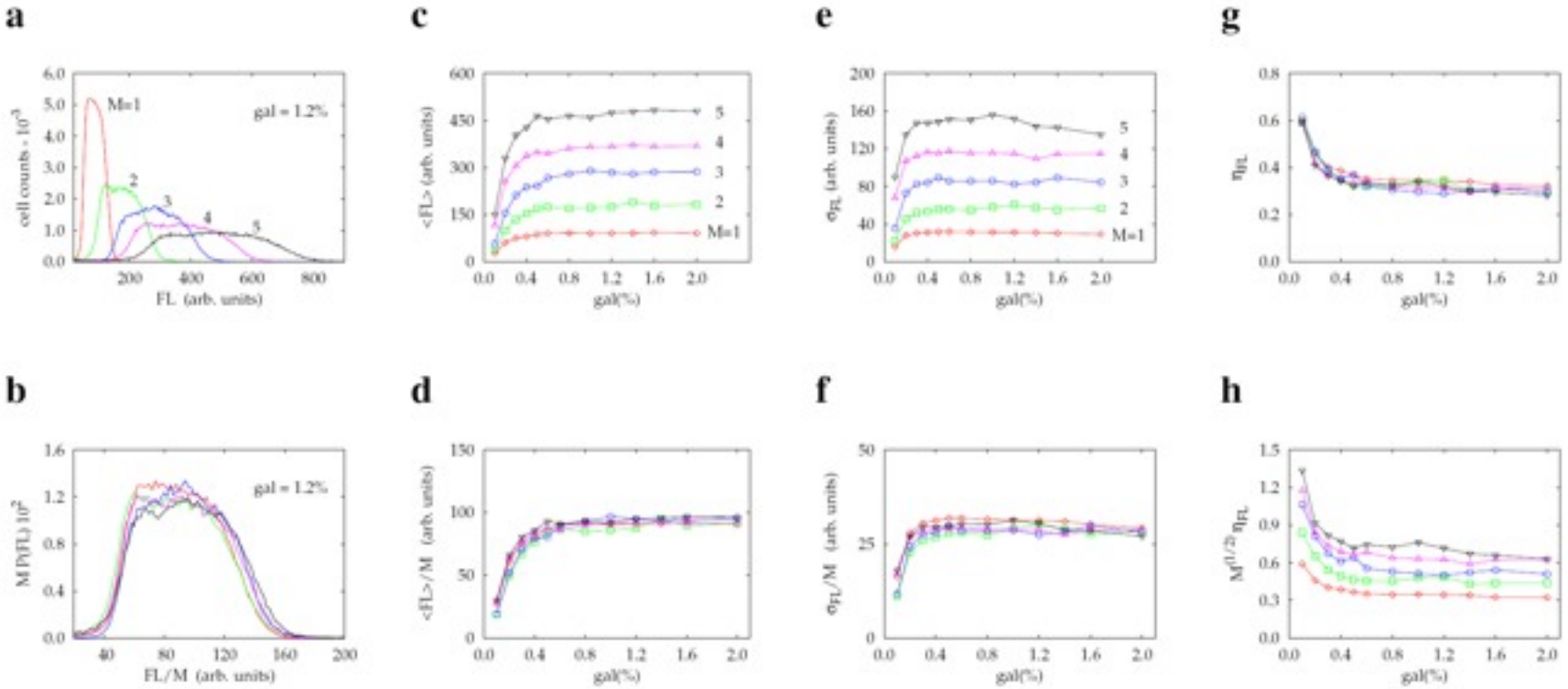
Volfson et al., Nature,2006

Intrinsic vs. Extrinsic noise



Volfson et al., Nature,2006

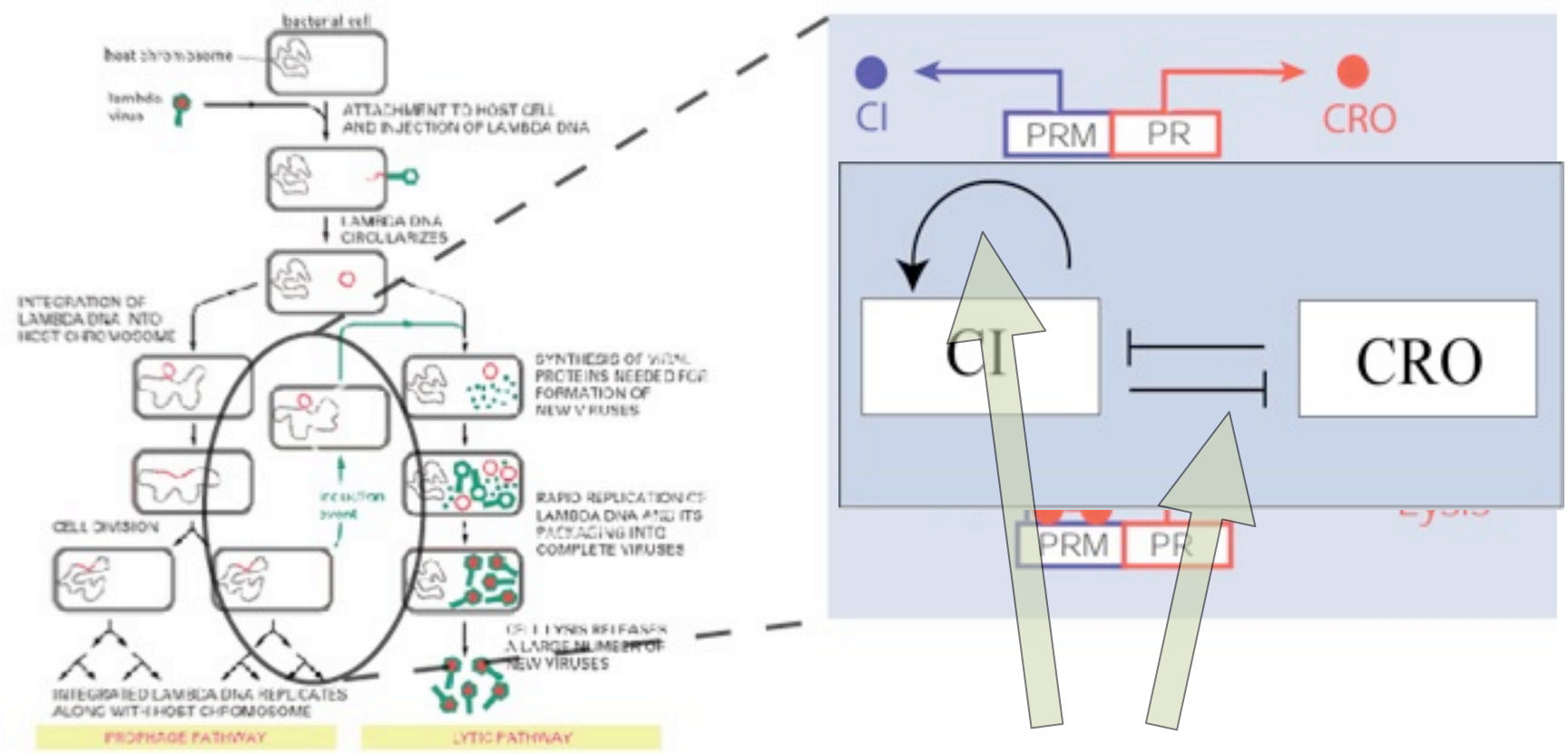
Flow cytometry data



Intrinsic noise is small in Yeast

Bistability: λ-Phage Life Cycle

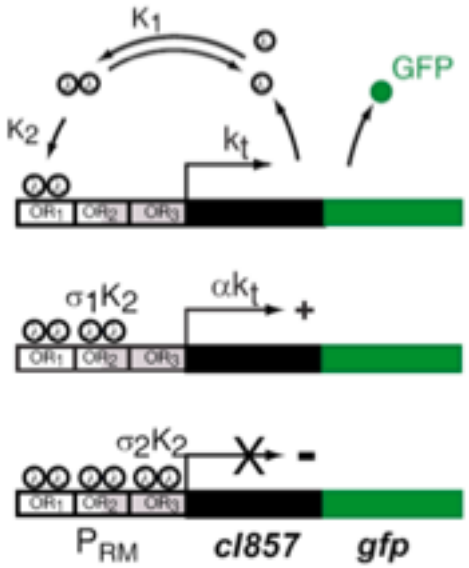
M.Ptashne, 2002

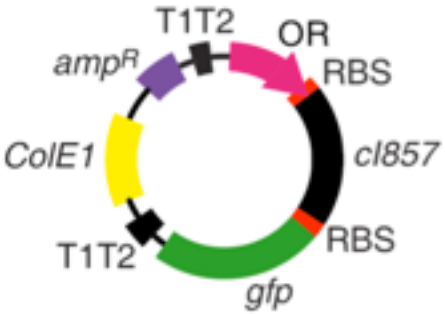


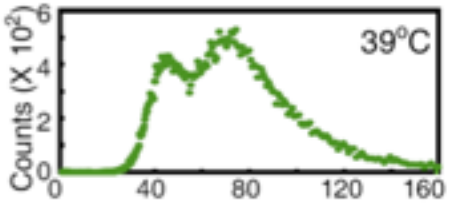
Two positive feedback loops

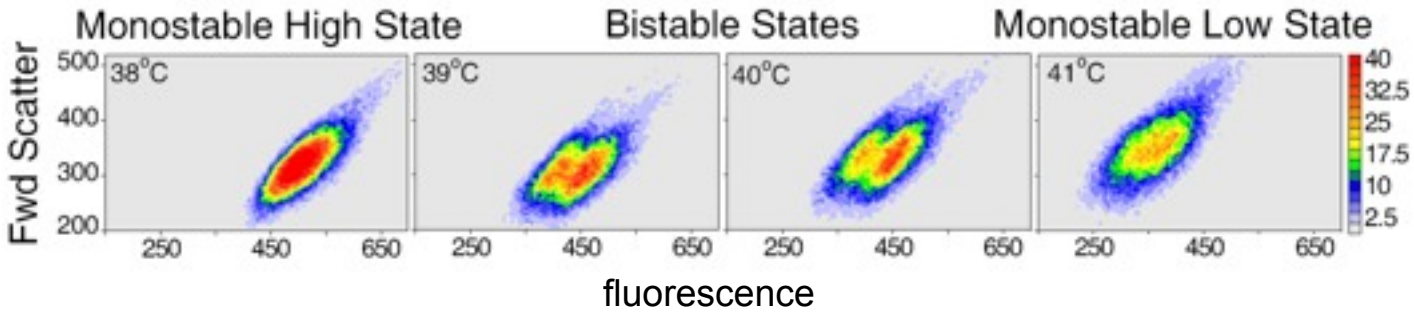
Genetic noise

Experiments with synthetic bistable autoregulatory circuit in *E. coli* (derived from λ-phage)







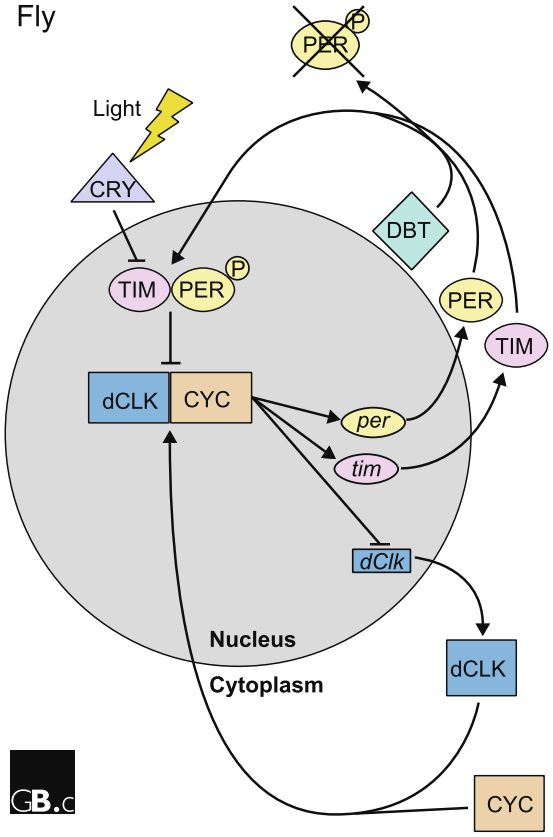


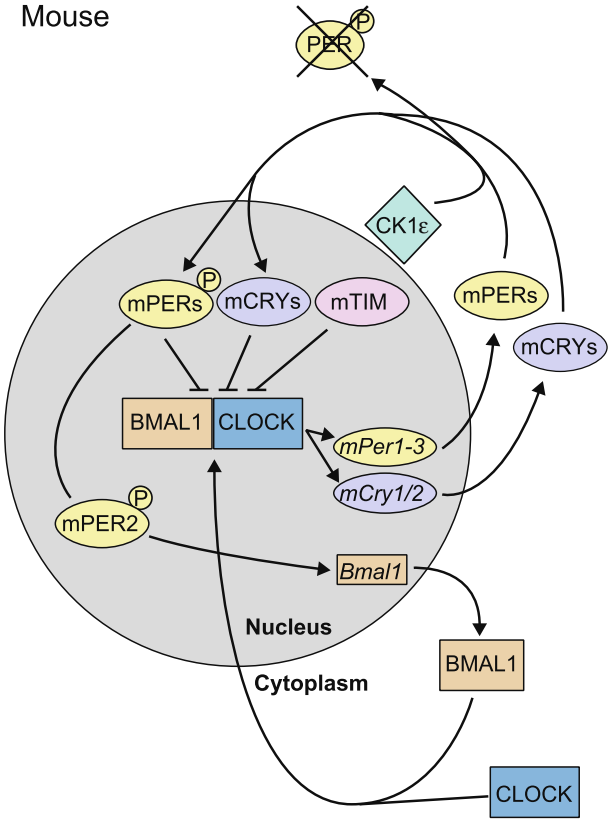
Isaacs et al., 2003

Oscillations: circadian clock

Neurospora crassa

Courtesy of Stu Brody

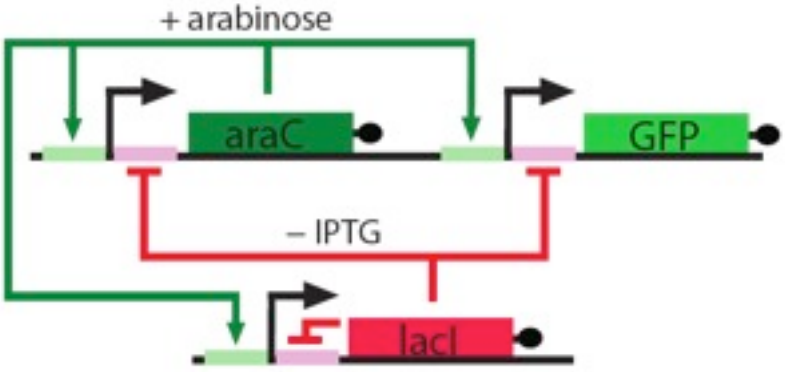




Synthetic gene oscillator in in *E.coli*

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

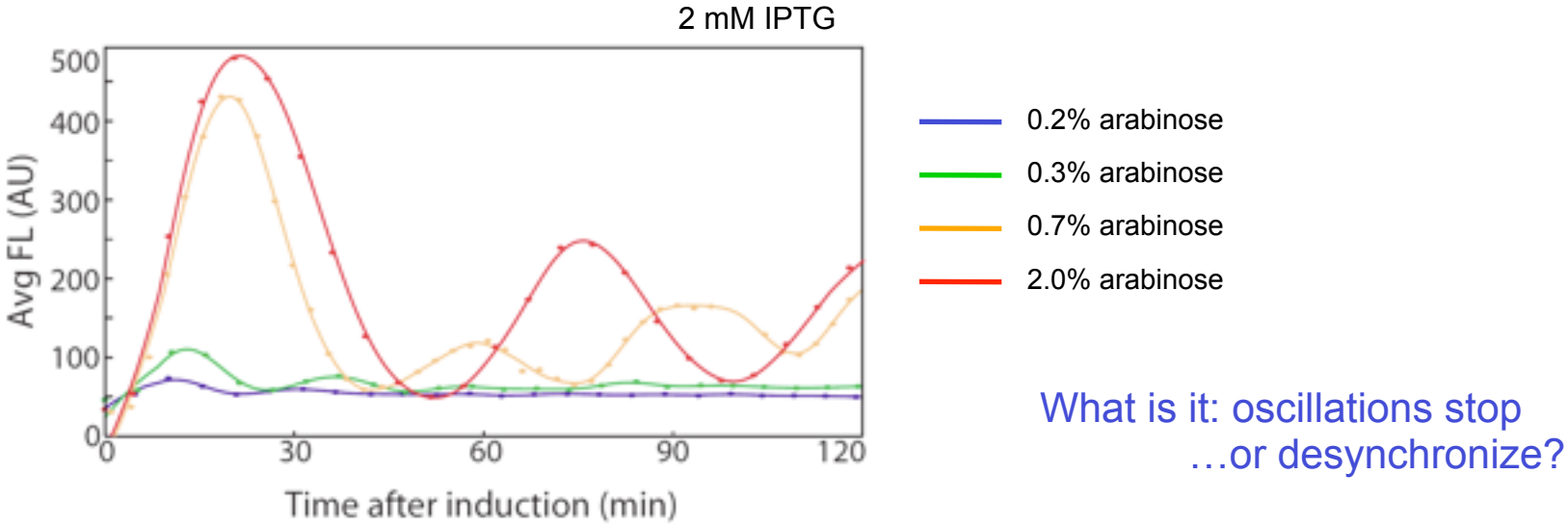
Design: use AraC as activator and LacI as repressor



- Three plasmid with identical promoters: (i) activator, (ii) repressor, (iii) reporter
- All proteins are tagged with ssRA tag for fast enzymatic decay (~15-20min)
- Two experimental "knobs": Arabinose required for activator binding and IPTG prohibits repressor binding

Flow cytometry:

time series of fluorescence in initially synchronized batch culture of cells

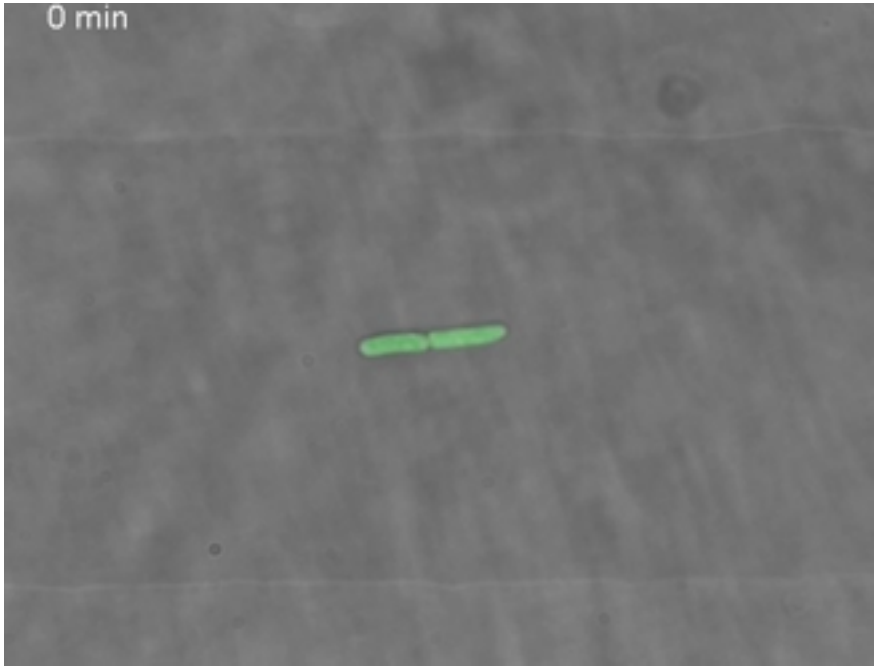


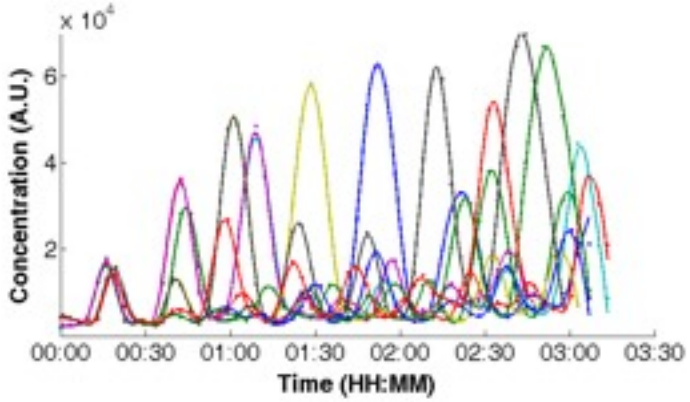
What is it: oscillations stop …or desynchronize?

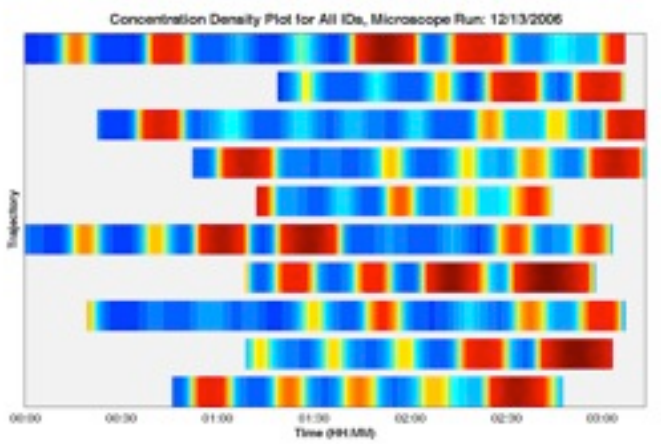
Single cell experiments

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

2mM IPTG
0.7% arabinose





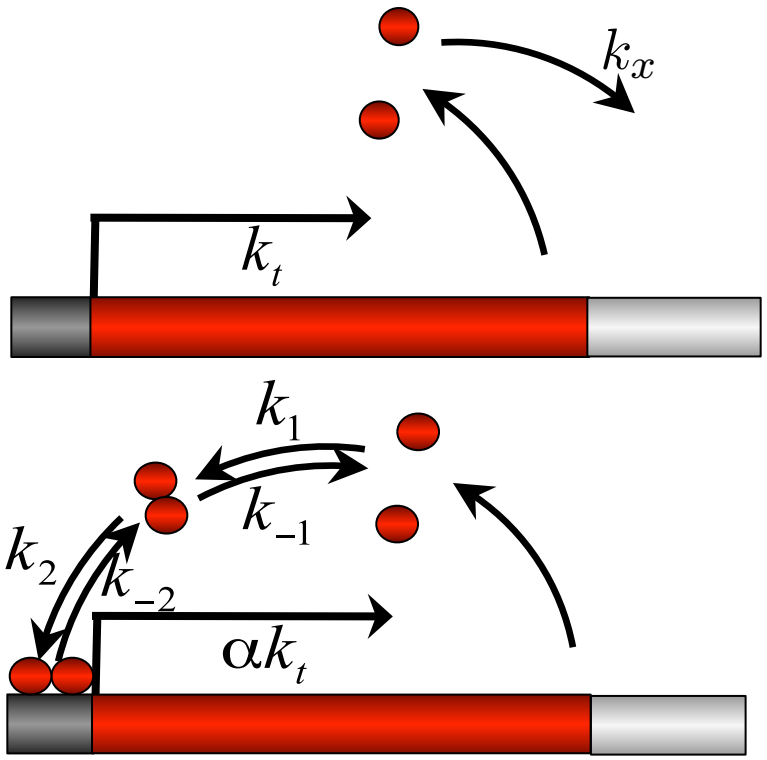


Modeling gene regulation

Techniques for modeling gene networks dynamics

- Boolean dynamics
- Deterministic Equations (Mass Action kinetics)
- Monte-Carlo Simulations; Master Equations
- Stochastic Differential Equations

Modeling gene regulation: Single gene circuit



Fast

$$X + X \xrightarrow{k_1} X_2$$
$$X_2 \xrightarrow{k_{-1}} X + X$$
$$D_0 + X \xrightarrow{k_2} D_1$$
$$D_1 \xrightarrow{k_{-2}} D_0 + X_2$$

Slow

$$D_0 + P \xrightarrow{k_t} D_0 + P + X$$
$$D_1 + P \xrightarrow{\alpha k_t} D_1 + P + X$$
$$X \xrightarrow{k_x} \emptyset$$

Binding/unbinding rate: ≤1 sec
Transcription rate: ~10^3 basepairs/min
Translation rate: ~10^2 aminoacids/min
mRNA degradation rate ~3min
Transport in/out of nucleus 10min..10hrs
Protein degradation 10min..hours

Mass action kinetics

Fast

$$X + X \xrightarrow{k_1} X_2$$
$$X_2 \xrightarrow{k_{-1}} X + X$$
$$D_0 + X \xrightarrow{k_2} D_1$$
$$D_1 \xrightarrow{k_{-2}} D_0 + X_2$$

Slow

$$D_0 + P \xrightarrow{k_t} D_0 + P + X$$
$$D_1 + P \xrightarrow{\alpha k_t} D_1 + P + X$$
$$X \xrightarrow{k_x} \emptyset$$

$$\dot{x} = -2k_1x^2 + 2k_{-1}x_2 - k_x x + k_t p_0(d_0 + \alpha d_1)$$
$$\dot{x}_2 = k_1x^2 - k_{-1}x_2 - k_2 d_0 x_2 + k_{-2}d_1$$
$$\dot{d}_0 = -k_2 d_0 x_2 + k_{-2}d_1$$
$$\dot{d}_1 = k_2 d_0 x_2 - k_{-2}d_1$$

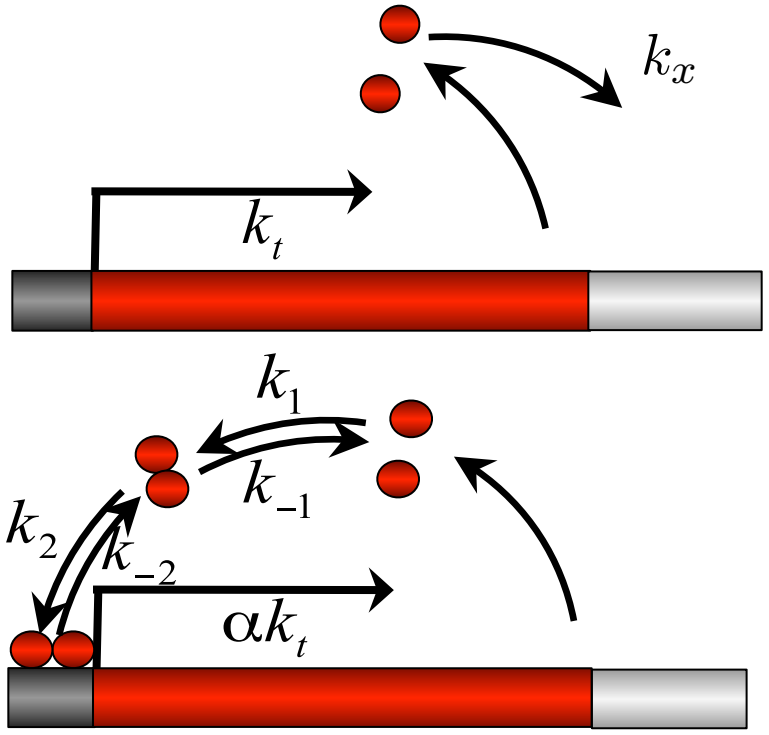
Separation of scales:

$$x_t = x + 2x_2 + 2d_1$$
$$d = d_0 + d_1$$
$$x_2 = K_1 x^2 \qquad K_1 = k_1/k_{-1}$$
$$d_1 = K_2 d_0 x_2 \qquad K_2 = k_2/k_{-2}$$

$$\dot{x}_t = \mathcal{P}_x \dot{x} = k_t p_0 d \frac{1 + \alpha K_1 K_2 x^2}{1 + K_1 K_2 x^2} - k_x x$$

$$\mathcal{P}_x = x_t'(x) = 1 + 4K_1 x + \frac{4dK_1 K_2 x^2}{1 + K_1 K_2 x^2}$$

Including fluctuations: Monte-Carlo Simulations



Jump probabilities:

$$P_1(2X \rightarrow X-2) = k_1 x(x-1)$$
$$P_2(X_2 \rightarrow 2X) = k_{-1} x_2$$
$$P_3(D_0 \rightarrow D_0 + X) = k_t d_0 x$$
$$P_4(D_1 \rightarrow D_1 + X) = \alpha k_t d_1 x$$
$$P_5(D_0 \rightarrow D_1) = k_2 d_0 x$$
$$P_6(D_1 \rightarrow D_0) = k_{-2} d_1$$
$$P_7(X \rightarrow) = k_x x$$

Simulations:

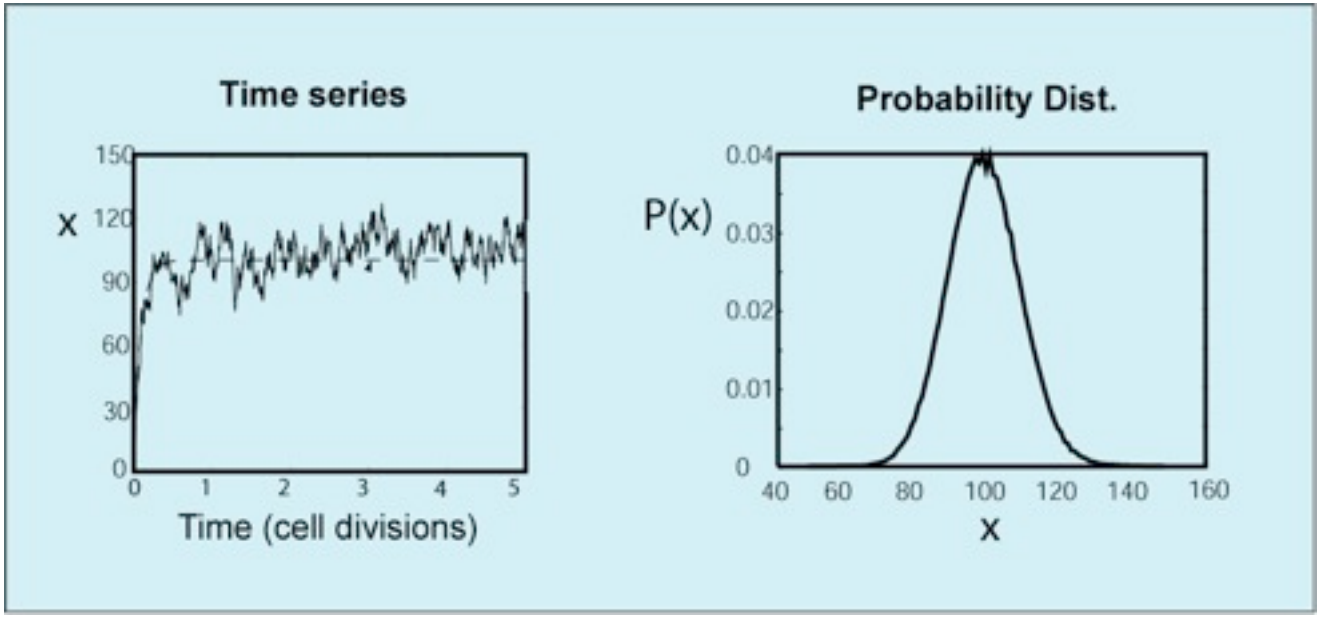
1. Use (x, x_2, d) to determine P_i
2. Determine τ
3. Determine specific reaction and update
4. Loop

Time between reactions:

$$\tau = (1/\sum_i P_i)\ \ln(1/r)$$

D. Gillespie, *J. Phys. Chem.* **81**:2340 (1977)

Monte Carlo Data



Complete and exact

Straightforward to simulate

Computationally expensive

Nonintuitive

Since it is exact, it is the gold standard for evaluating the accuracy of other modeling approaches

Master equation description

n – total # of monomers; u – # of unbound dimers; b - # of bound dimers

Master equation for $p_{n,u,b}$

$$\begin{aligned}\frac{dp_{n,u,b}}{dt} &= k_x[(n+1-2u-2b)p_{n+1,u,b} - (n-2u-2b)p_{n,u,b}] \\ &+k_t(d-b+\alpha b)[p_{n-1,u,b} - p_{n,u,b}] \\ &+k_1[(n-2u-2b+2)(n-2u-2b+1)p_{n,u-1,b} \\ &-(n-2u-2b)(n-2u-2b-1)p_{n,u,b}] \\ &-k_{-1}[(u+1)p_{n,u+1,b} - dp_{n,u,b}] \\ &+k_{-2}[(b+1)p_{n,u-1,b+1} - bp_{n,u,b}] - k_2[u(d-b)p_{n,u,b} - (u+1)(d-b+1)p_{n,u+1,b-1}]\end{aligned}$$

Projection: $p_{n,u,b} = p_n p_{u,b|n}$

$$\begin{aligned}\frac{dp_n}{dt} &= k_x[(n+1-2\langle u|n+1\rangle - 2\langle b|n+1\rangle)p_{n+1} - (n-2\langle u|n\rangle - 2\langle b|n\rangle)p_n] \\ &+k_t[(d+(\alpha-1)\langle b|n-1\rangle)p_{n-1} - (d+(\alpha-1)\langle b|n\rangle)p_n]\end{aligned}$$

$$p_{u,b|n} = p_0 \frac{K_1^{u+b} K_2^b d!n!}{b!(d-b)!u!(n-2u-2b)!}$$

$$\langle u \mid n\rangle = K_1 m(m-1); \quad \langle b \mid n\rangle = \frac{dK_1K_2m(m-1)}{1+K_1K_2m(m-1)}$$

$$m = n - 2\langle u \mid n\rangle - 2\langle b \mid n\rangle$$

See Kepler & Elston, Biophys. J., 2001

Back to ODE

In the continuum limit (large n): Fokker-Planck equation

$$\frac{\partial \rho(x_t)}{\partial t} = \frac{\partial}{\partial x_t}\left[\left(k_x x - k_t d\frac{1+\alpha K_1 K_2 x^2}{1+K_1 K_2 x^2}\right)\rho\right] + \frac{1}{2}\partial^2_{x_t}\left[\left(k_x x + k_t d\frac{1+\alpha K_1 K_2 x^2}{1+K_1 K_2 x^2}\right)\rho\right]$$

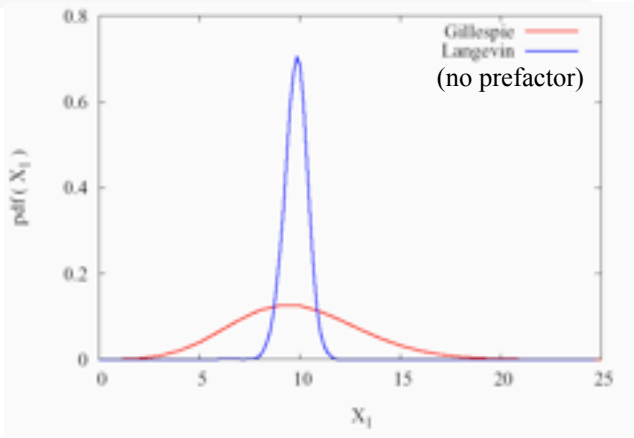
$$x_t = x + 2K_1 x^2 + \frac{2dK_1 K_2 x^2}{1+K_1 K_2 x^2}$$

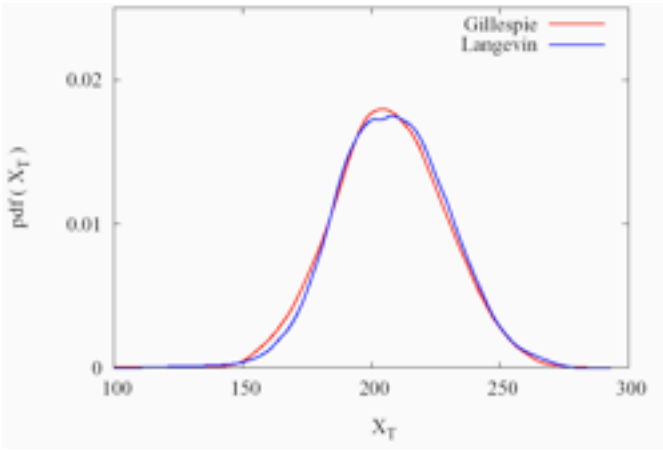
Corresponding Langevin equation

$$\frac{dx_t}{dt} = k_t d\frac{1+\alpha K_1 K_2 x^2}{1+K_1 K_2 x^2} - k_x x + \sqrt{D(x_t)}\xi$$

$$D(x) = k_x x + k_t d\frac{1+\alpha K_1 K_2 x^2}{1+K_1 K_2 x^2}$$

with $x = x(x_t)$

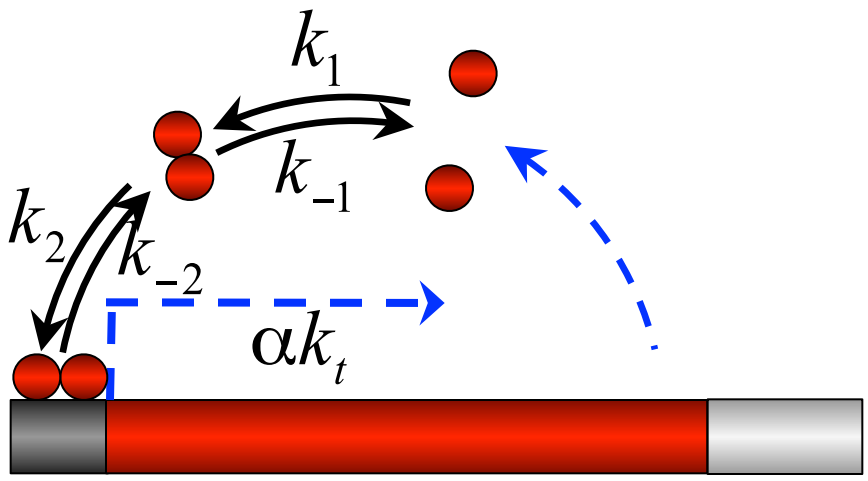




Fast reaction noise is filtered out

Kepler & Elston, *Biophys J* **81**:3116 (2001)

Transcriptional delay



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$$X_2 \xrightarrow{k_{-1}} X + X$$
$$D_0 + X \xrightarrow{k_2} D_1$$
$$D_1 \xrightarrow{k_{-2}} D_0 + X_2$$

$$\dot{x} = -2k_1x^2 + 2k_{-1}x_2 - k_x x + k_t p_0(d_0(t-T) + \alpha d_1(t-T))$$
$$\dot{d}_0 = -k_2 d_0 x_2 + k_{-2} d_1$$
$$\dot{d}_1 = k_2 d_0 x_2 - k_{-2} d_1$$
$$\dot{x}_2 = k_1 x^2 - k_{-1} x_2 - k_2 d_0 x_2 + k_{-2} d_1$$

[cf. Santillán & Mackey, 2001]

Slow

$$X \xrightarrow{k_x} \emptyset$$

Delayed

$$D_0 + P \xrightarrow{k_t} D_0 + P + X$$
$$D_1 + P \xrightarrow{\alpha k_t} D_1 + P + X$$

After projection

$$\mathcal{P}_x \dot{x} = k_t p_0 d \frac{1 + \alpha K_1 K_2 x^2(t-T)}{1 + K_1 K_2 x^2(t-T)} - k_x x$$

Genetic oscillations: Hopf bifurcation

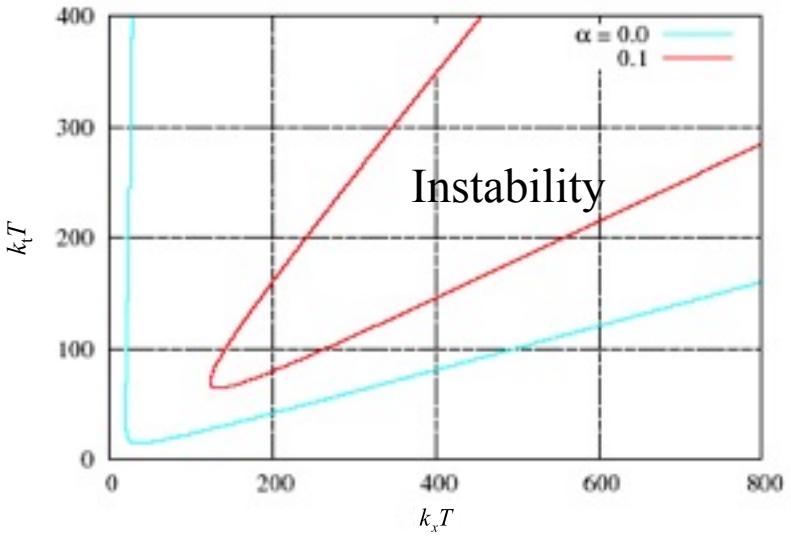
Fixed point:

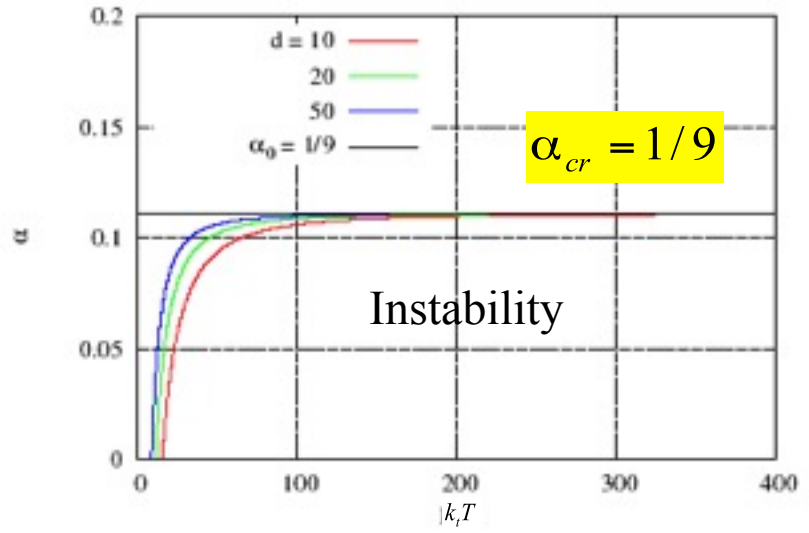
$$0 = -k_x \bar{x} + k_t d H(\bar{x}) \qquad H(\bar{x}) = \frac{2K_1K_2(\alpha - 1)}{(1 + K_1K_2\bar{x}^2)^2}$$

Complex eigenvalues

$$\mathcal{P}_x(\bar{x})\lambda = -k_x + k_t d G(\bar{x}) e^{-\lambda T} \qquad G(\bar{x}) = \frac{dH(\bar{x})}{d\bar{x}}$$

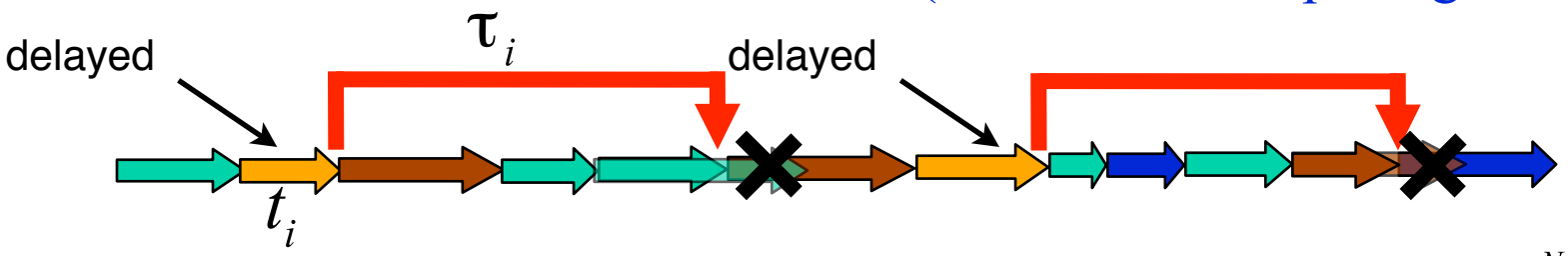
$$d = 10,\ K_1 = K_2 = 1$$





Stochastic simulations of delayed reactions

(modified Gillespie algorithm)



N species $\mathbf{X}$, M independent reactions μ rates a_μ stoichiometric matrix $\mathbf{S} \in \mathbb{Z}^{N \times M}$

Markovian statistics of reaction times:

- exponential "next reaction" time distribution

$$P(t) \propto \exp\left[-t \sum_\mu a_\mu\right]$$

- which reaction to choose?

$$P(\mu = \mu') = a_{\mu'} / \sum_\mu a_\mu$$

- immediate execution

$$\mathbf{X} \longrightarrow \mathbf{X} + \mathbf{S}_{\mu'}$$

Delayed reactions [e.g. transcription, translation]:

- Delayed execution
- Execution time τ : fixed or stochastic, e.g. with Gaussian time distribution

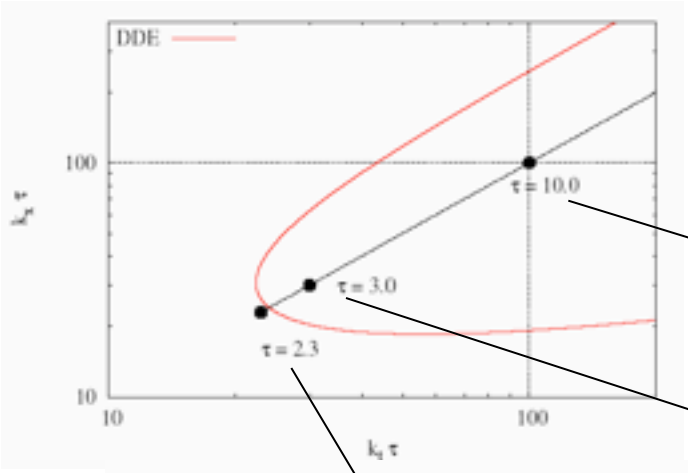
$$P_\mu(\tau) \propto \exp\left[-(\tau - \tau_0)^2 / \sigma_\mu^2\right]$$

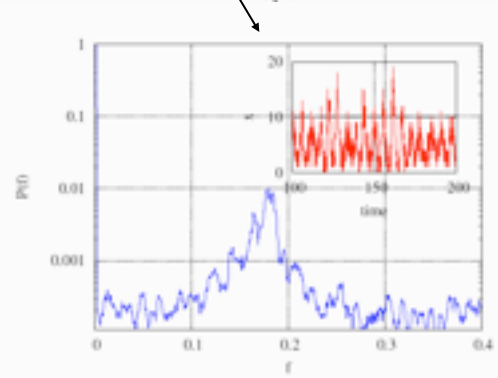
Bratsun, Volfson, LT, Hasty, *PNAS*, 2005

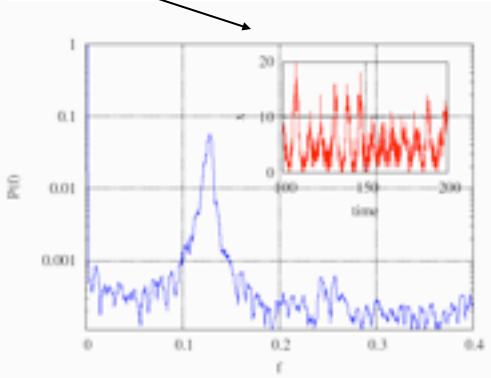
Stochastic simulations

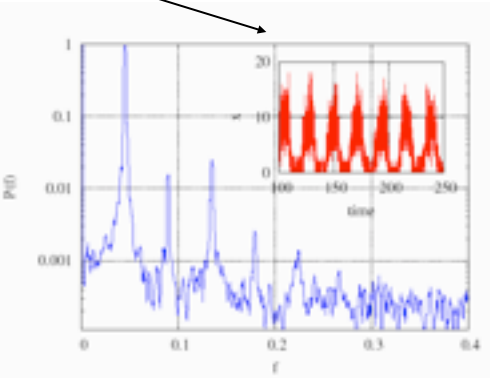
Bratsun, Volfson, LT, Hasty, *PNAS*, 2005

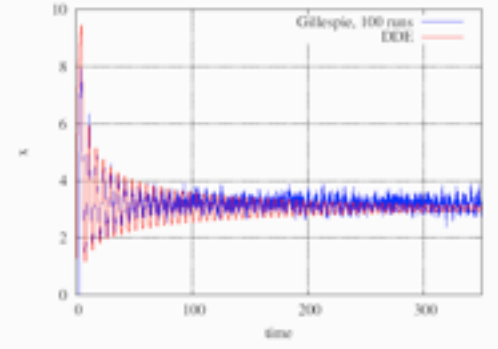
$$d = 10, k_1 = k_2 = 100, K_1 = K_2 = 0.5, \alpha = 0$$

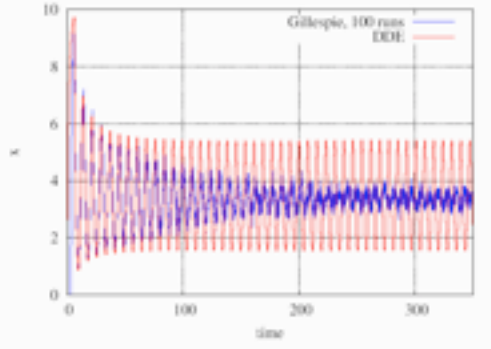


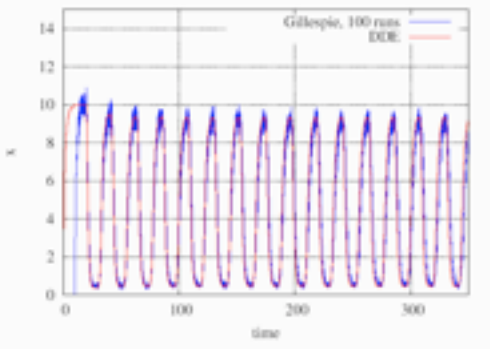












Synthetic bacterial oscillator: A detailed model

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

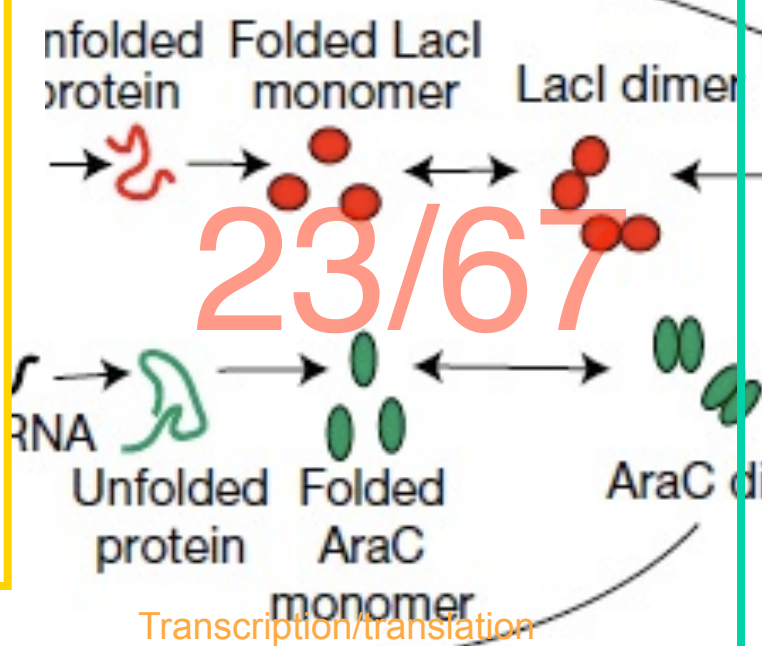
$$P_{1,j}^{a,r} \xrightarrow{f(X)} P_{0,j}^{a,r}$$

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

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

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

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



Regulation

Transcription/translation

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

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

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

Enzymatic decay

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

Explicit delay model

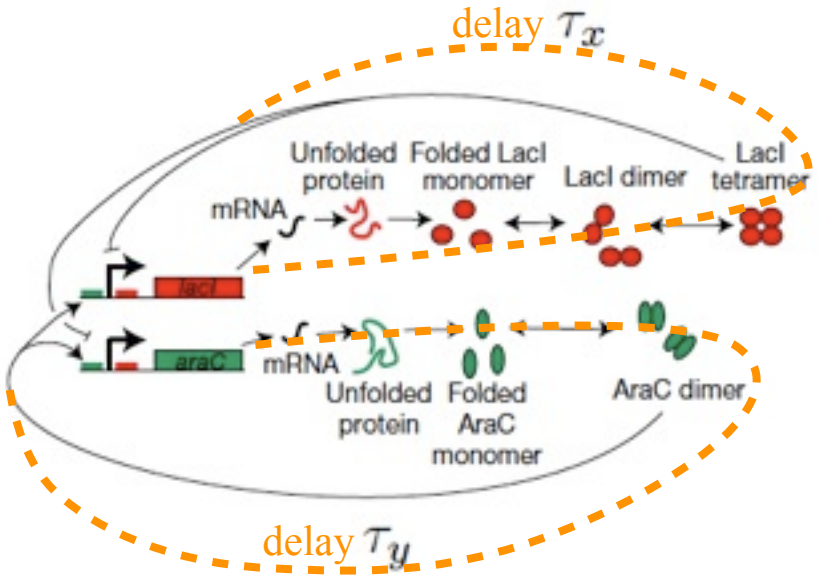
replace chains of interactions with a single effective delay in feedback

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



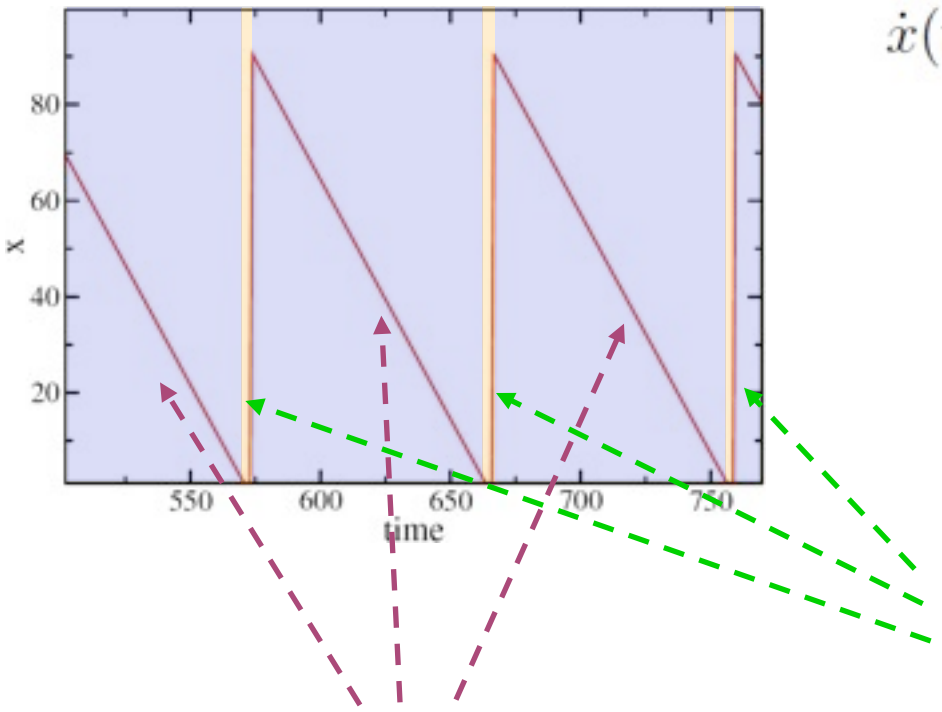
$$\frac{dx}{dt} = \frac{\alpha(1+f y_{\tau_y}^2/C_{0y}^2)}{(1+x_{\tau_x}^2/C_{0x}^2)(1+y_{\tau_y}^2/C_y^2)} - \frac{\delta_x x}{C_{1x}+x}$$

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

Delayed auto-repression: 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

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

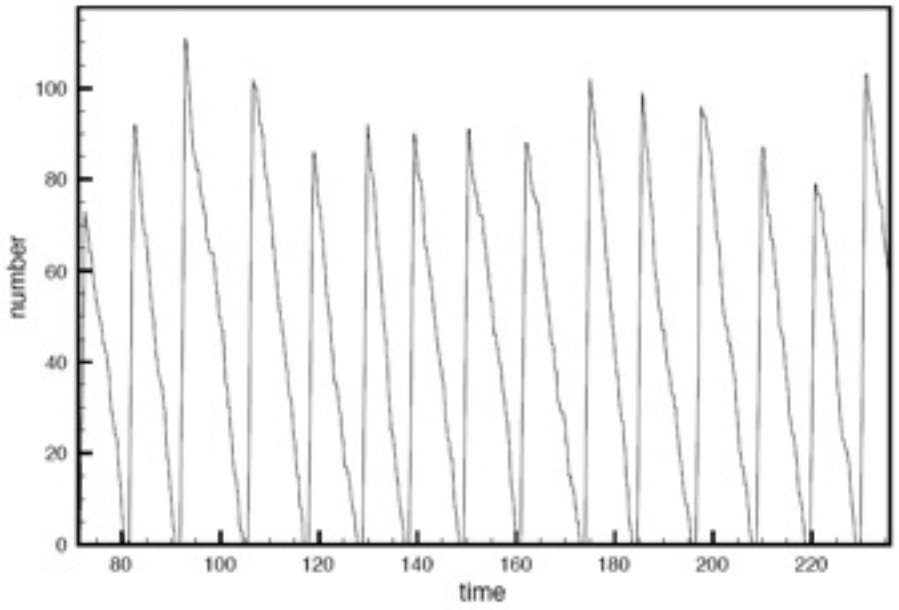
- Two time scales: relaxation oscillations

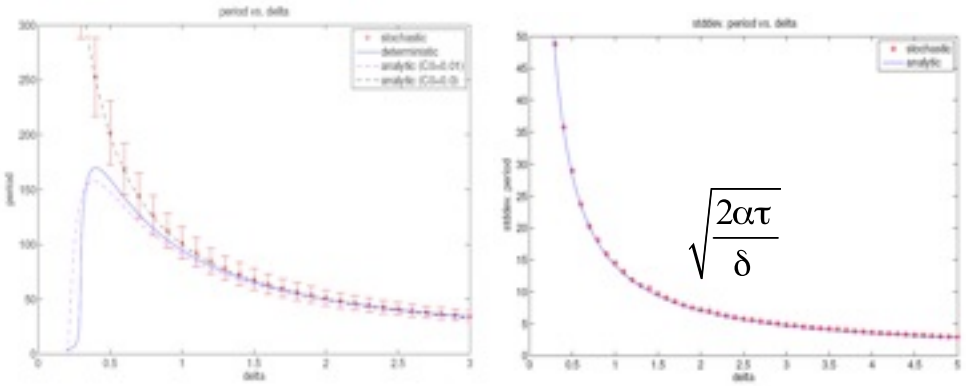
Period:

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

Zeroth-order degradation: stochastic model

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





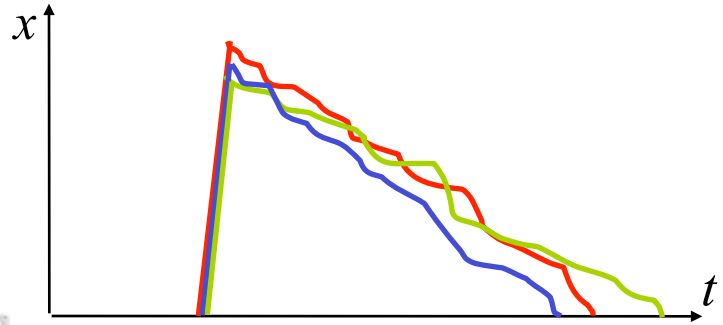
$$\sqrt{\frac{2\alpha\tau}{\delta}}$$

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

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

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

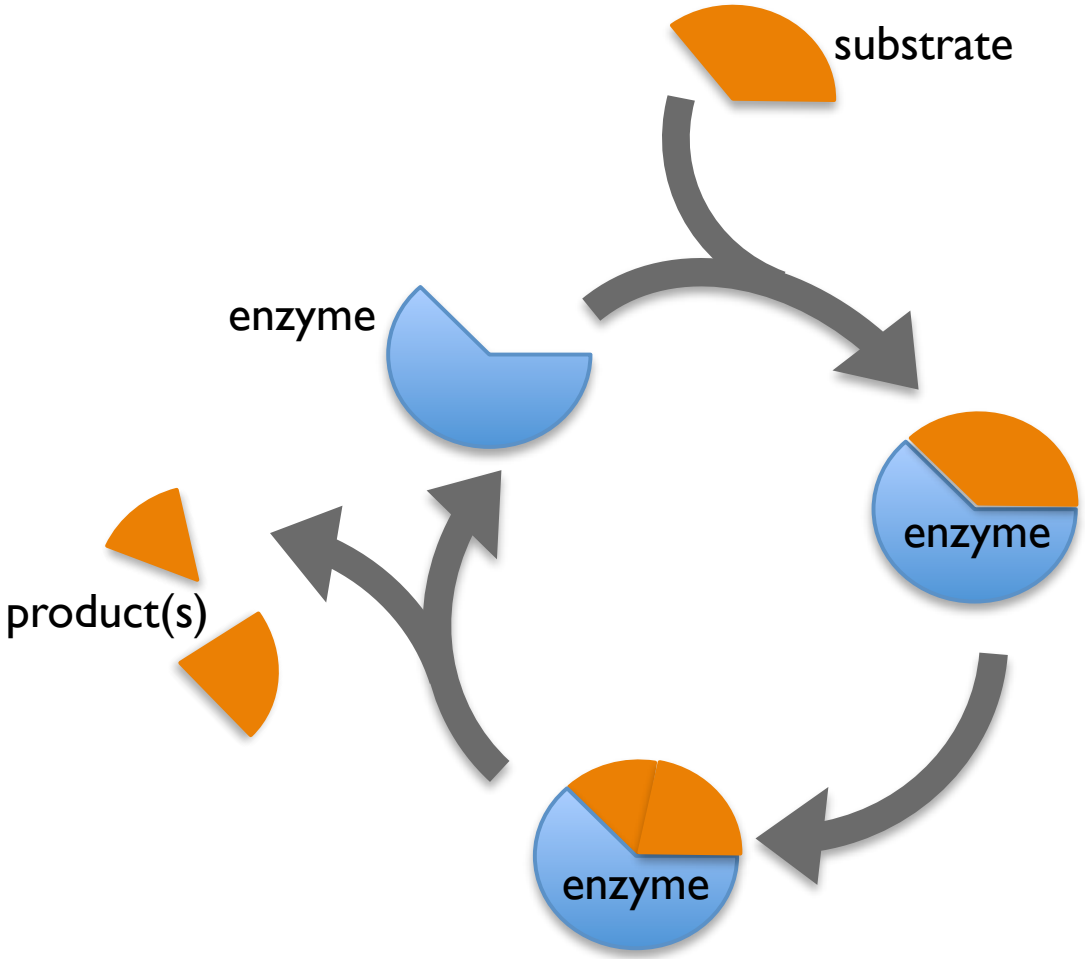
$$v_d = \left\langle T_d \right\rangle = \left\langle x_{\max} \right\rangle / \delta = \alpha\tau / \delta$$

Period variance:

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

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

Enzymatic processing



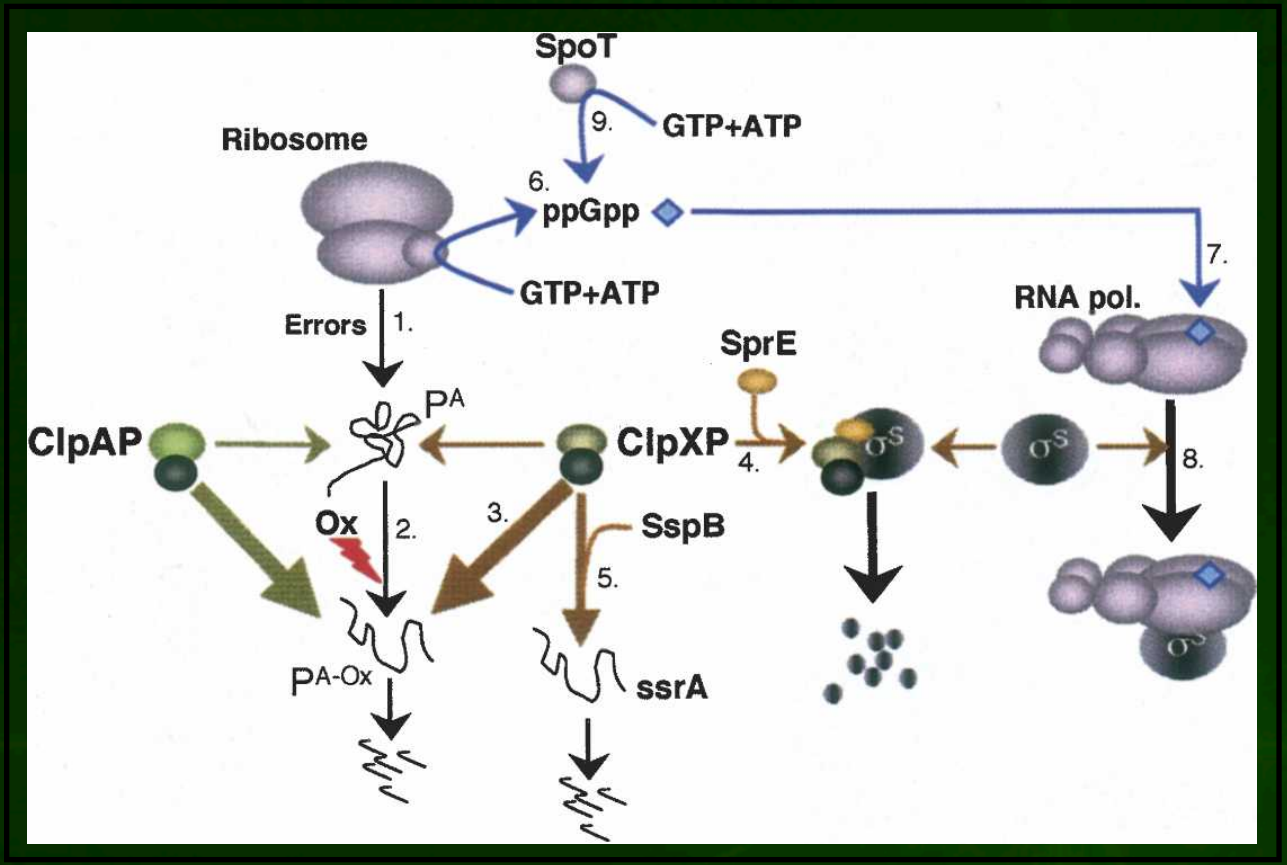
$$E + S \xrightarrow{k_1} [ES]$$

$$[ES] \xrightarrow{k_{-1}} E + S$$

$$[ES] \xrightarrow{k_2} E + P$$

Examples: metabolism, transcription, translation, degradation, signaling...

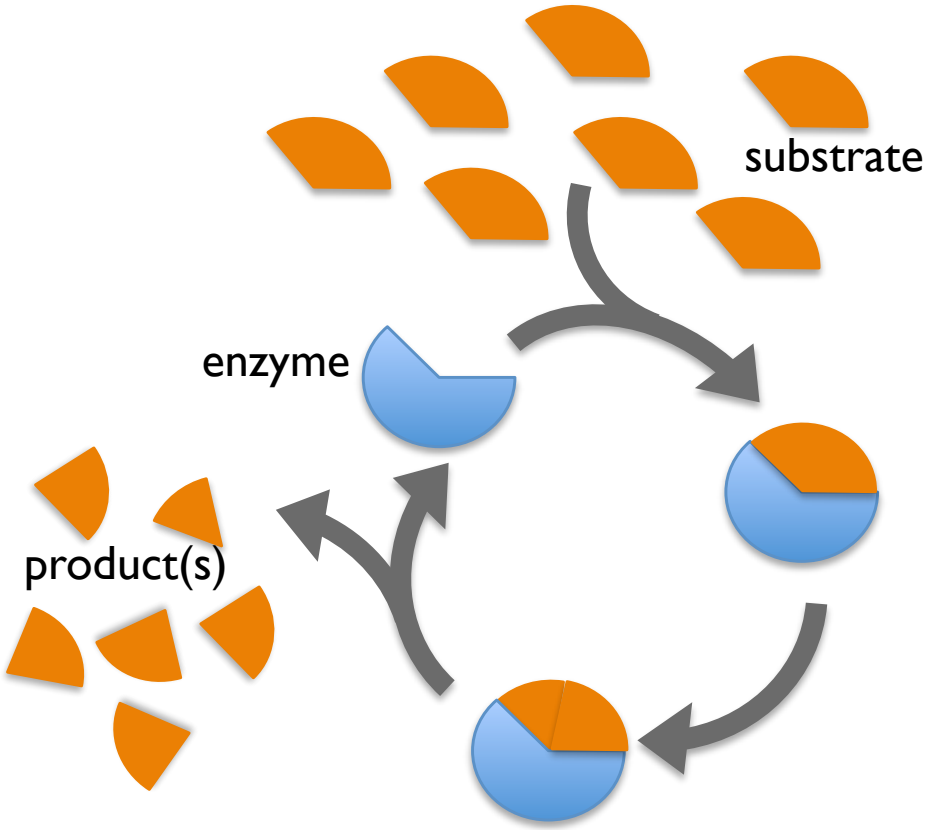
Enzymatic Queueing: ClpXP degradation machine



ClpXP senses proteins that are tagged for fast degradation and destroys them

normally unstable proteins are stabilized and become active when ClpXP is overloaded

Enzymatic queueing



Lots of substrate: queues!

How do fluctuations affect queueing?

Two Cases

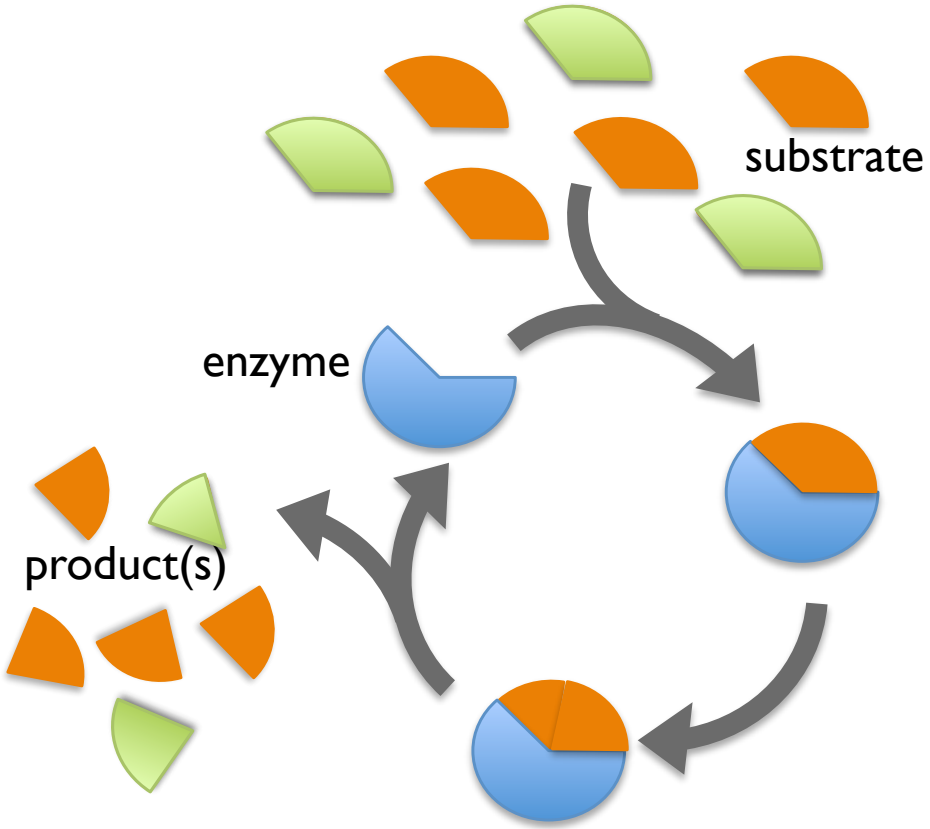
Underloaded

- service rate > incoming traffic rate
- little competition between customers
- queue lengths are short

Overloaded

- service rate < incoming traffic rate
- competition between customers
- queue lengths are long

Enzymatic queueing: multiple substrates



$$E + S_i \xrightarrow{k_1} [ES_i]$$

$$[ES_i] \xrightarrow{k_{-1}} E + S_i$$

$$[ES_i] \xrightarrow{k_2} E + P_i$$

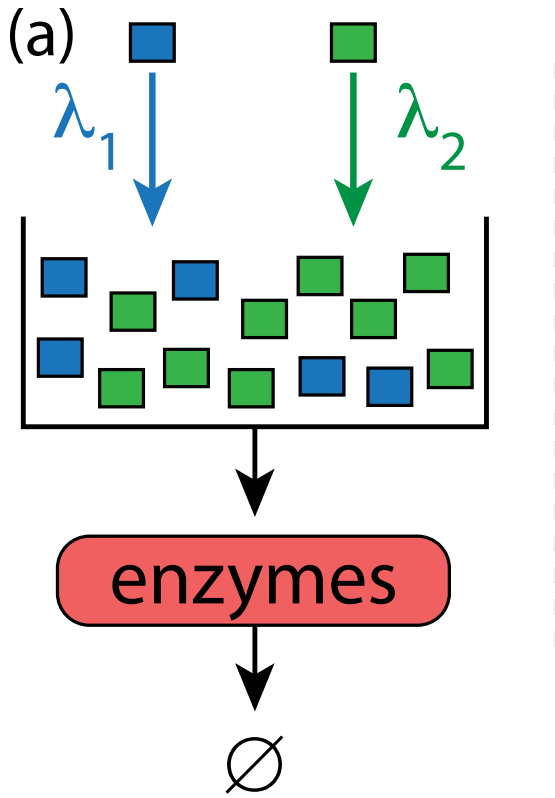
Stochastic kinetics:

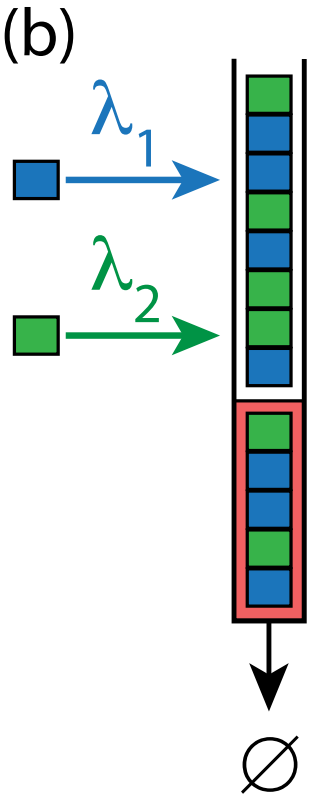
fluctuating substrate

fluctuating products

...correlations?

Queueing Model





$$D_i \xrightarrow{\lambda_i} X_i + D_i,$$

$$X_i + E \underset{\eta_-}{\overset{\eta_+}{\rightleftharpoons}} X_i E,$$

$$X_i E \xrightarrow{\mu} E,$$

$$X_i E \xrightarrow{\gamma} E,$$

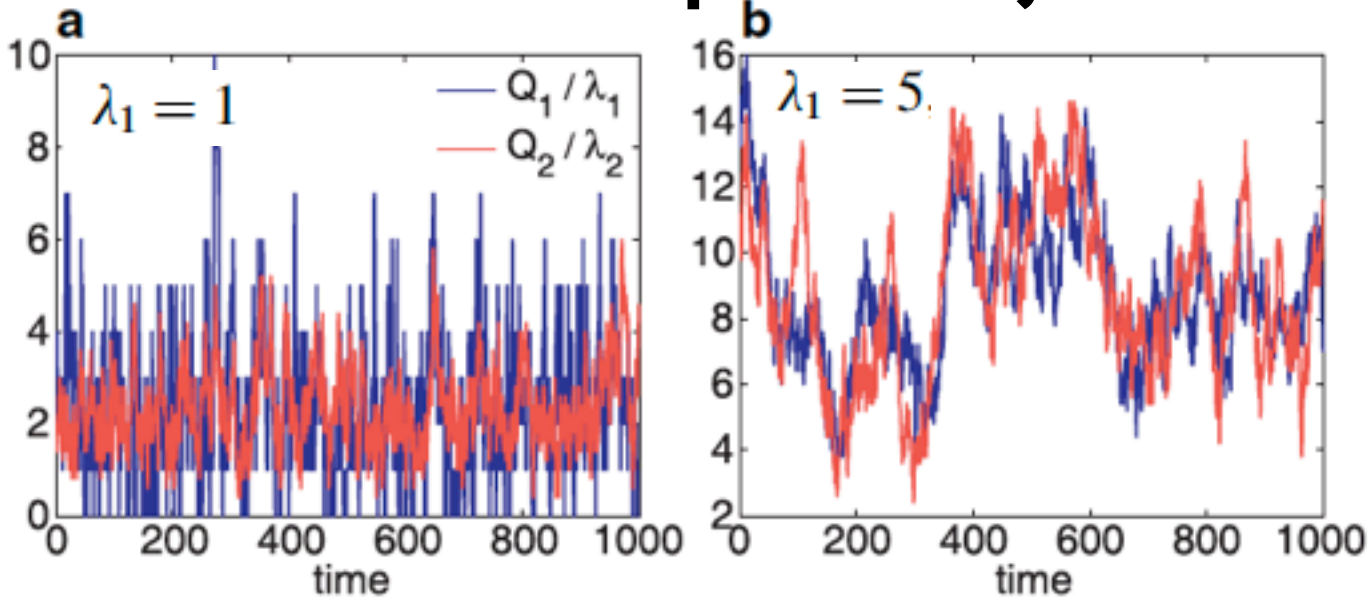
$$X_i \xrightarrow{\gamma} \varnothing.$$

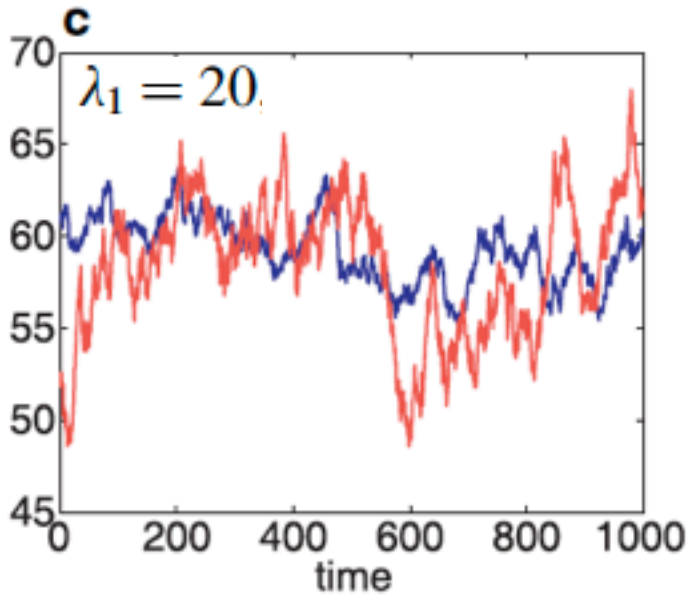
$Q_i(t)$ # of molecules of type *i*

$N(t) = \sum_{i=1}^{m} Q_i(t)$

$K = \eta_-/\eta_+$

Sample trajectories





$\lambda_2 = 5$, $\mu = 1$, $\gamma = 0.01$,

$\eta_+ = 20$, and $\eta_- = 100$

number of enzymes $L = 10$

Main result

Conditioned on the total number of protein molecules N in the system being n, the steady-state distribution for Q is a multinomial distribution with parameters $(n; p_1,\ldots,p_m)$, where $p_i = \lambda_i/\Lambda$, $i = 1,\ldots,m$, and $\Lambda = \sum_{i=1}^{m} \lambda_i$

$$P(Q = (q_1, \ldots, q_m)) = P(N = n)\frac{n!}{q_1!\ldots q_m!}\prod_{i=1}^{m} p_i^{q_i}$$

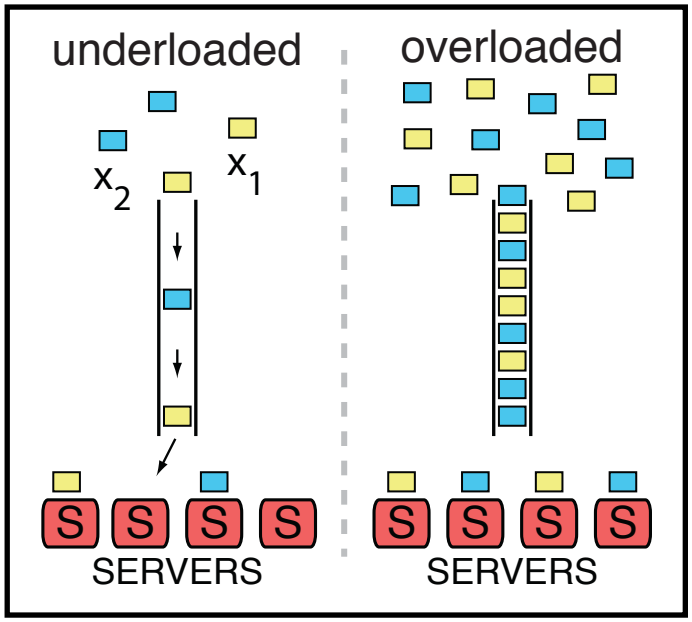
$$P(N = n) = c\frac{\Lambda^n}{\prod_{\ell=1}^{n}\phi(\ell)},\ n = 0, 1, 2, \ldots, \qquad \phi(n) = \min(n, L)\mu + n\gamma$$

$$n = \sum_{i=1}^{m} q_i,\ \ N = \sum_{i=1}^{m} Q_i. \qquad \nu(N) = Var(N)/E[N] \text{ is the Fano factor for } N$$

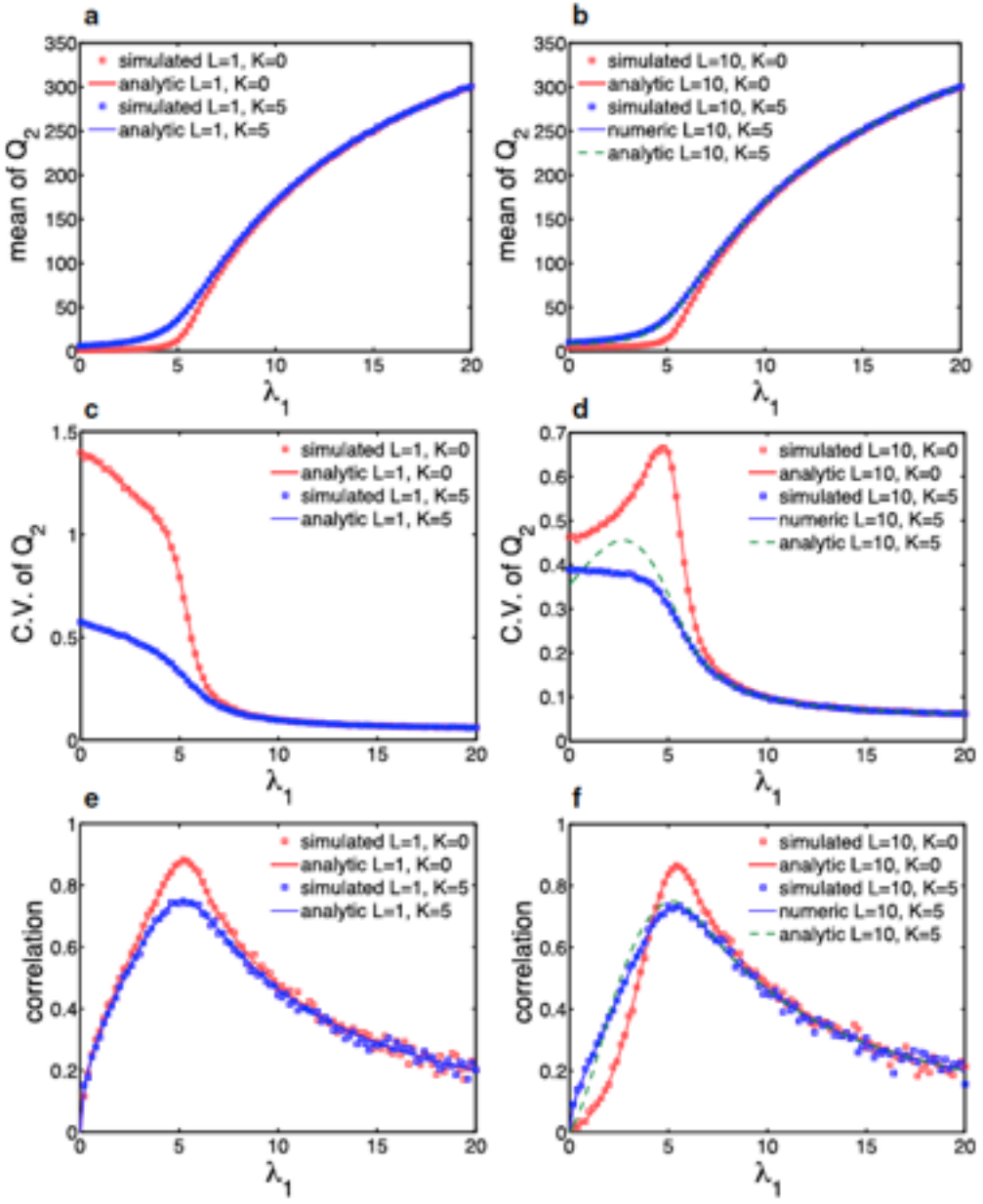
Correlation:

$$r_{ij} = \frac{\nu(N) - 1}{\left(\nu(N) - 1 + \frac{1}{p_i}\right)^{1/2}\left(\nu(N) - 1 + \frac{1}{p_j}\right)^{1/2}}$$

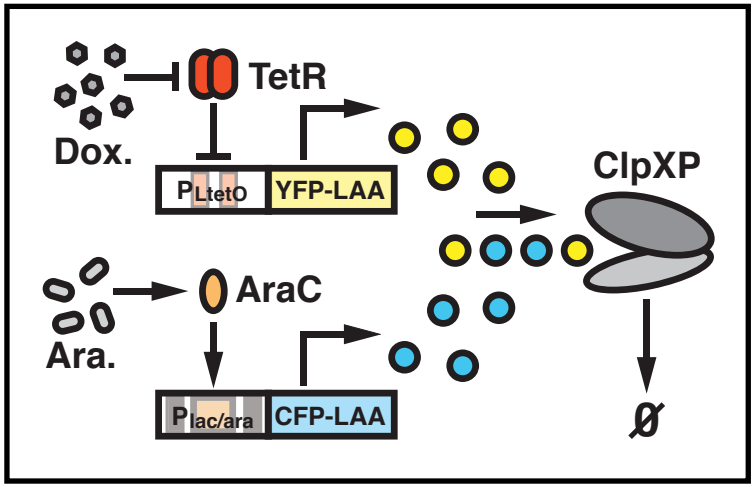
Queueing Model



$$K = \eta_-/\eta_+$$

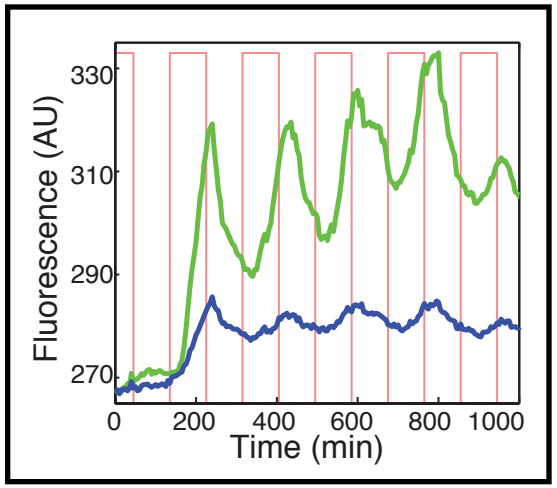


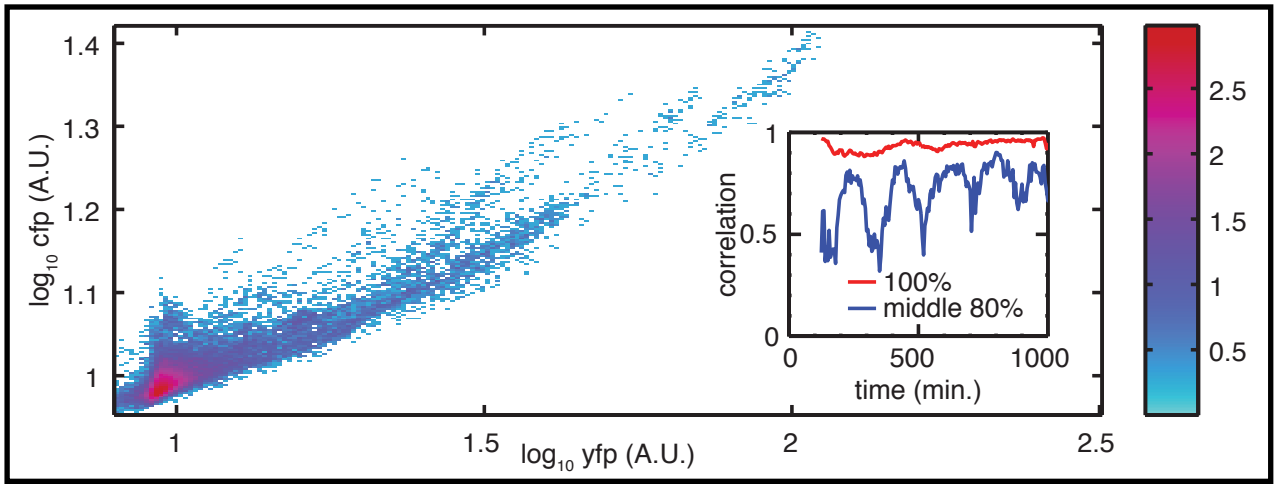
Queueing in experiment: ClpXP degradation



on a microfluidic device, we drove direct induction (green) by a time-dependent chemical signal

time-dependent signal is carried through





periodic noise resonance associated with crossing through balance

Conclusions

- Genetic regulation is strongly affected by fluctuations, both intrinsic and extrinsic
- In many cases, extrinsic factors dominate
- Theoretical description of extrinsic variability is developed and compared with experimental data from multiple promoter-gene pairs.
- Deterministic and stochastic description of regulatory delays developed, delays of transcription/translation of auto-repressor may lead to increased fluctuations levels and oscillations even when deterministic model shows no Hopf bifurcation
- Modified Gillespie algorithm is developed for simulating time-delayed reactions

- D. Volfson, J. Marciniak, N. Ostroff, L. Tsimring, J. Hasty, origins of extrinsic variability in eukaryotic gene expression, Nature, 439, 861-864 (16 Feb 2006).
- D.A. Bratsun, D.N. Volfson, L.S. Tsimring, and J. Hasty, Delay-induced stochastic oscillations in gene regulation. Proc. Natl. Acad. Sci., **102**, no.41, 14593-12598 (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)
- W. Mather, N. A. Cookson, J. M. Hasty, L. S. Tsimring , and R. J. Williams. Correlation resonance generated by coupled enzymatic processing. Biophys. J., 2010, **99**, 3172-3181