Topic 4: Genetic Circuits

A. Integrated model of gene expression
   1. constitutive gene expression
   2. transcription control
   3. translation control and mRNA stability
   4. control of protein degradation

B. Simple circuits using only transcriptional control
   1. negative autoregulation
   2. positive autoregulation
   3. toggle switch
   4. oscillators

C. Noise in gene expression

D. Metabolic control
   1. gene regulation
   2. effect of inducer
   3. metabolic feedback

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C. Genetic noise

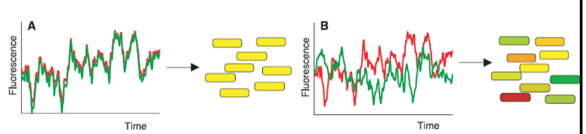
• extrinsic: variation of "external factors", e.g., RNAp, ribosome, temp, …
• intrinsic: stochasticity in mRNA and protein synthesis, TF-DNA binding,…
➔ cell-to-cell variability if noise is amplified by feedback
➔ escape from one state to another within a single cell

Q: fraction of total noise from extrinsic/intrinsic sources?

Stochastic Gene Expression in a Single Cell

Michael B. Elowitz,[1,2*] Arnold J. Levine,[1] Eric D. Siggia,[2] Peter S. Swain[2]

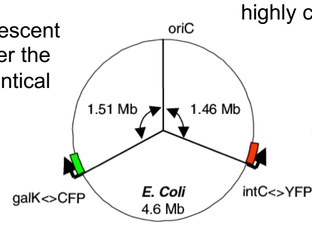
SCIENCE VOL 297 16 AUGUST 2002



HIGH extrinsic noise and LOW intrinsic noise: highly correlated variations

LOW extrinsic noise and HIGH intrinsic noise: uncorrelated fluctuations

Put two fluorescent proteins under the control of identical promoters.



$$\eta_{ext}^2 = \frac{\langle cy\rangle - \langle c\rangle\langle y\rangle}{\langle c\rangle\langle y\rangle} \qquad \eta_{\text{int}}^2 = \frac{\langle (c-y)^2\rangle}{2\langle c\rangle\langle y\rangle}$$

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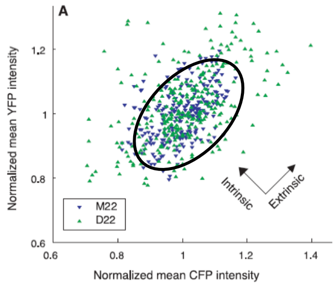
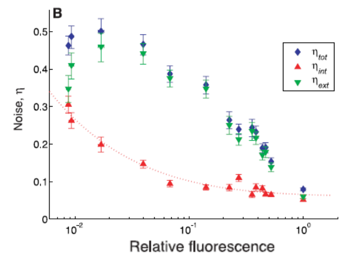
Scatter plot of mean fluorescence intensity/cell for many cells.



Population average at different inducer concentrations



➔ extrinsic noise larger, but intrinsic noise not negligible

➔ noise decreases with expression levels

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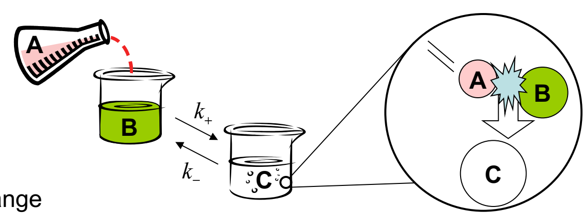
Intrinsic stochasticity

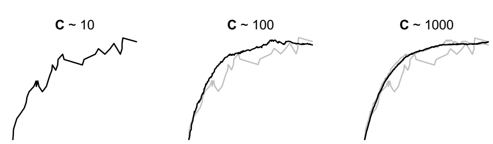
- rate equation:

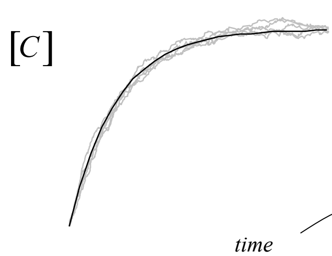
$$\frac{d[C]}{dt} = k_+[A][B] - k_-[C]$$

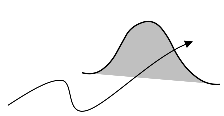
Continuous, differential change in the concentration of **C**



Large no. molecules in **C** ~ 10^{23} (mole)







What effect do these fluctuations have on the observable dynamics of the system?

➔ use *Master Equations* to characterize these fluctuations

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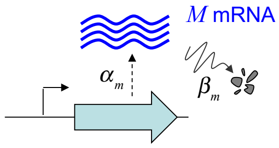
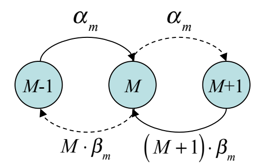
Consider mRNA synthesis



two reactions:

$$M \xrightarrow{\alpha_m} M+1$$
$$M \xrightarrow{M \cdot \beta_m} M-1$$



corresponding rate equation: $\dfrac{dM}{dt} = \alpha_m - \beta_m \cdot M$

in term of mRNA conc $m \equiv M / V$: $\dfrac{dm}{dt} = \alpha_m / V - \beta_m \cdot m$

Describe discrete dynamics by $P(M,t)$
– probability to find M mRNA molecules at time t.

Write a probability conservation equation:

$$\frac{\partial P}{\partial t} = \underbrace{\left\{\alpha_m \cdot P(M-1,t) + (M+1)\cdot \beta_m \cdot P(M+1,t)\right\}}_{\text{Flux In}} - \underbrace{\left\{\alpha_m \cdot P(M,t) + M \cdot \beta_m \cdot P(M,t)\right\}}_{\text{Flux Out}}$$

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$$\frac{\partial P}{\partial t} = \left\{\alpha_m \cdot P(M-1,t) + (M+1)\cdot \beta_m \cdot P(M+1,t)\right\} - \left\{\alpha_m \cdot P(M,t) + M \cdot \beta_m \cdot P(M,t)\right\}$$
$$= \alpha_m \cdot \left[P(M-1,t) - P(M,t)\right] + \beta_m \cdot \left[(M+1)\cdot P(M+1,t) - M \cdot P(M,t)\right]$$

➔ Solve for $P(M,t)$ by z-transform: $F(z,t) = \sum_{M=0}^{\infty} z^M P(M,t)$

Multiply by z^M, then sum over all M, get

$$\frac{\partial F}{\partial t} = \alpha_m \cdot (z-1) \cdot F(z,t) - \beta_m \cdot (z-1) \cdot \frac{\partial F}{\partial z}$$

Turns a discrete-differential equation for P into a partial differential equation for F

At steady-state, $\dfrac{\partial F}{\partial t} = 0 \quad \Rightarrow F^*(z) = \exp\left[\dfrac{\alpha_m}{\beta_m}(z-1)\right]$

some properties of $F(z,t)$:

$$F(z,t)\big|_{z=1} = \sum_{M=0}^{\infty} P(M,t) = 1$$

Avg[M]

$$\frac{\partial}{\partial z} F(z,t)\bigg|_{z=1} = \sum_{m=0}^{\infty} M \cdot P(M,t) = \langle M \rangle$$

$$\frac{\partial^2}{\partial z^2} F(z,t)\bigg|_{z=1} = \langle M^2 \rangle - \langle M \rangle$$

$\langle M \rangle = \dfrac{\alpha_m}{\beta_m}$, and $\text{var[M]} = \dfrac{\alpha_m}{\beta_m}$

➔ avg = var signature of Poisson

Fano Factor: $\dfrac{\text{var}[M]}{\langle M \rangle} = \dfrac{\alpha_m / \beta_m}{\alpha_m / \beta_m} = 1$

rel. fluctuation: $\eta = \sqrt{\dfrac{\text{var}[M]}{\langle M \rangle^2}} = \dfrac{1}{\sqrt{\langle M \rangle}}$

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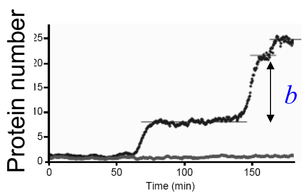
Next include translation

No. mRNA: M; No. proteins: N

rate equations:

$$\frac{dM}{dt} = \alpha_m - \beta_m \cdot M$$

$$\frac{dN}{dt} = \alpha_p \cdot M - \beta_p \cdot N$$



Cai, Friedman, Xie (2006) *Nature* **440**: 358.

write out the Master equation governing $P(M,N,t)$.

solve by double z-transform: $\langle N \rangle = \alpha_m \cdot \alpha_p / \beta_m \cdot \beta_p$

Fano factor: $\frac{\mathrm{var}[N]}{\langle N \rangle} = 1 + \frac{\alpha_p}{\beta_m + \beta_p}$

$\approx 1 + \frac{\alpha_p}{\beta_m}$ since $\beta_m \gg \beta_p$

Translational Bursting b
≈ average number of proteins translated within mRNA lifetime

- Moment generating functions *only work if the transition rates are constant or linear* (e.g., α_m and $M{\cdot}\beta_m$)
- For regulated networks, e.g., autoactivator with synthesis rate $\alpha_m \bullet \mathcal{G}(N/V)$, need to use approximations…

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Approximation 1: Numerical simulation

Common method: Gillespie's stochastic simulation algorithm.

[Gillespie (1977) *J. Chem. Phys.* **81**: 2340.]

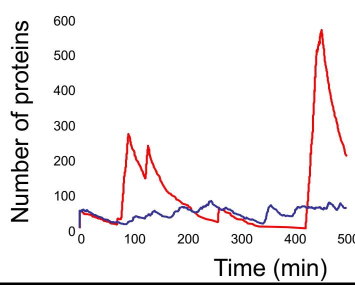
- Use the transition rate to compute a probability distribution for *when the next reaction will be completed*
- Use the transition rates to compute a probability distribution for *which reaction will occur*
- Update the state for each species of reactant

➔ easy to program, but computes one trajectory at a time; no deep insight.

Apply to constitutive protein synthesis

$$\dot{M} = \alpha_m - \beta_m \cdot M$$

$$\dot{N} = \alpha_p \cdot M - \beta_p \cdot N$$



Parameter	Blue	Red
α_m (min^{-1})	0.1	0.01
β_m	1/5	1/5
α_p	2	20
β_p	1/50	1/50
<N>	50	50
b	10	100

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Apply to the autoactivator model

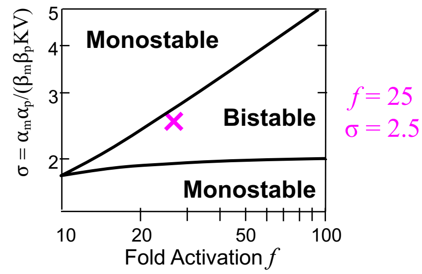
rate equations:

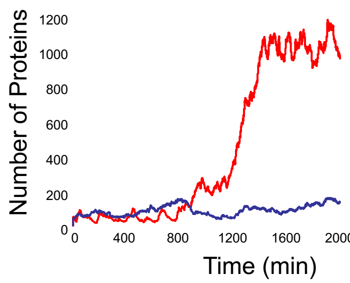
$$\dot{M} = \alpha_m \cdot \mathcal{G}(A/KV) - \beta_m \cdot M$$

$$\dot{A} = \alpha_p \cdot M - \beta_p \cdot A$$

$$\text{with } \mathcal{G} = \frac{f^{-1} + \left(A/KV\right)^n}{1 + \left(A/KV\right)^n}.$$

deterministic phase diagram





Parameter	Blue	Red
α_m (min^{-1})	25	2.5
α_p	0.2	2
<A>	1250	1250
b	1	10

➔ no longer 'bistable' for large burstiness

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Approximation 2: Langevin dynamics

Consider constitutive protein expression:

- add a Gaussian "noise" $\xi(t)$ to the deterministic rate equation

$$\dot{N} = \alpha - \beta \cdot N(t) + \xi(t), \quad \alpha \equiv \alpha_m \alpha_p / \beta_m, \quad \beta \equiv \beta_p$$

- adjust the variance of $\xi(t)$, D, to match the Fano factor

$$\left\langle \xi(t) \right\rangle = 0, \quad \left\langle \xi(t)\xi(t') \right\rangle = 2D\delta(t - t')$$

- distribution of N evolves according to the Fokker-Planck equation

$$\frac{\partial}{\partial t} P(N,t) = -\frac{\partial}{\partial N}\left[\left(\alpha - \beta \cdot N\right) \cdot P\right] + D \cdot \frac{\partial^2}{\partial N^2} P$$

➔ solve for the steady-state distribution $P^*(N) \propto e^{-\frac{\beta}{2D}(N - \alpha/\beta)^2}$

$$\left\langle N \right\rangle = \alpha / \beta, \quad \text{var}[N] = \left\langle N^2 \right\rangle - \left\langle N \right\rangle^2 = D/\beta$$

$$\text{Fano factor: } \frac{\text{var}[N]}{\left\langle N \right\rangle} = 1 + b \quad \Rightarrow \quad D = (1 + b) \cdot \alpha$$

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Apply to the autoactivator: $\dot{A} = \alpha \cdot \mathcal{G}(A/KV) - \beta \cdot A, \quad \text{with } \mathcal{G}(x) = \frac{f^{-1} + x^n}{1 + x^n}$

- add Gaussian "noise" $\xi(t)$ to the deterministic rate equation

$$\dot{A} = \underbrace{\alpha \cdot \mathcal{G}(A/KV) - \beta \cdot A}_{f(A)} + \xi(t) \quad \text{with} \quad \langle \xi(t)\xi(t') \rangle = 2\underbrace{(1+b)\alpha \mathcal{G}(A/KV)}_{g(A)}\delta(t-t')$$

➔ amplitude of $\xi(t)$ depends on A: multiplicative noise

- Fokker-Planck equation for stochastic processes with multiplicative noise:

$$\frac{\partial}{\partial t} P(A,t) = -\frac{\partial}{\partial A}\left[f(A) \cdot P\right] + \frac{\partial^2}{\partial A^2}\left[g(A) \cdot P\right]$$

[c.f. Ito vs Stratanovich]

➔ solve for the steady-state distribution $P^*(A)$

$$f(A) \cdot P^*(A) = \tfrac{d}{dA} g(A) \cdot P^*(A) + g(A) \cdot \tfrac{d}{dA} P^*(A)$$

$$\ln P^*(A) = \int^A dA' \left[\frac{f(A') - \frac{d}{dA} g(A')}{g(A')}\right]$$

$$= \int^A dA' \frac{f(A')}{g(A')} - \ln g(A)$$

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Apply to the autoactivator: $\dot{A} = \alpha \cdot \mathcal{G}(A/KV) - \beta \cdot A, \quad \text{with } \mathcal{G}(x) = \frac{f^{-1} + x^n}{1 + x^n}$

- add Gaussian "noise" $\xi(t)$ to the deterministic rate equation

$$\dot{A} = \underbrace{\alpha \cdot \mathcal{G}(A/KV) - \beta \cdot A}_{f(A)} + \xi(t) \quad \text{with} \quad \langle \xi(t)\xi(t') \rangle = 2\underbrace{(1+b)\alpha \mathcal{G}(A/KV)}_{g(A)}\delta(t-t')$$

$$\ln P^*(A) = \int^A dA' \frac{f(A')}{g(A')} - \ln g(A) \qquad \sigma \equiv \alpha / (\beta K V)$$

$$= \text{const.} - \ln \mathcal{G}(A/KV) - \frac{KV}{1+b} \int^{A/KV} dx \left[\frac{x}{\sigma \cdot \mathcal{G}(x)} - 1\right]$$

$$\Rightarrow \quad P^*(A) \propto \frac{1}{\mathcal{G}(A/KV)} \exp\left\{ -\frac{KV}{1+b} \underbrace{\int^{A/KV} dx \left[\frac{x}{\sigma \cdot \mathcal{G}(x)} - 1\right]}_{\text{effective potential } U(A/KV)} \right\}$$

Probability being in the high state reduced f-fold

effective temperature = $(1+b)/(KV)$

➔ eff. temp increased by burstiness (b), decreased by No. proteins (KV)

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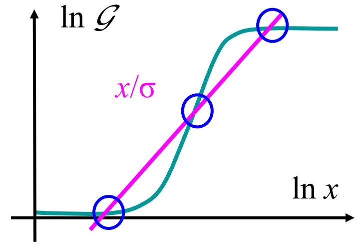
Effective potential $U(x) = \int^x dx' \left[\frac{x'}{\sigma \cdot \mathcal{G}(x')} - 1 \right]$, with $\mathcal{G}(x) = \frac{f^{-1} + x^n}{1 + x^n}$

$$\frac{dU}{dx} = \frac{x}{\sigma \cdot \mathcal{G}(x)} - 1 \xrightarrow{\left.\frac{dU}{dx}\right|_{x^*} = 0} \sigma \cdot \mathcal{G}\left(x^*\right) = x^*$$

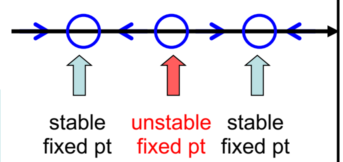
c.f. deterministic eqn: $\beta^{-1}\, \dot{x} = \sigma \cdot \mathcal{G}(x) - x$

➔ extrema of U(x) ⇔ fixed points

$$\left.\frac{d^2U}{dx^2}\right|_{x^*} = x^* \cdot \left(1 - s^*\right), \quad \text{where } s^* = \left.\frac{d \ln \mathcal{G}}{d \ln x}\right|_{x^*}$$



stability analysis (graphical):



➔ unstable fixed points (s*>1): maxima of U(x)
➔ stable fixed points (s* < 1): minima of U(x)

➔ robustness of bistability to stochastic fluctuations: compare “barrier height” to eff temperature

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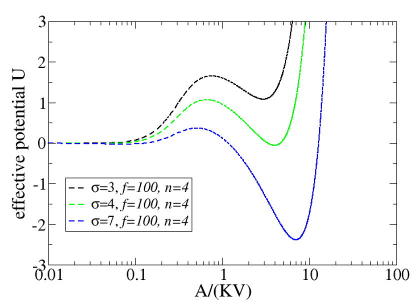
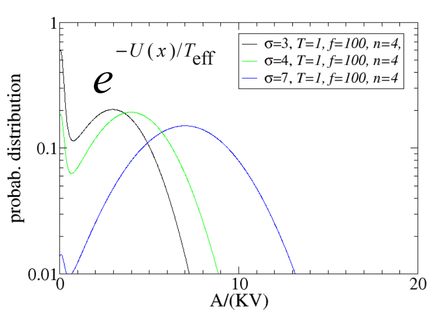
Effective potential $U(x) = \int^x dx' \left[\frac{x'}{\sigma \cdot \mathcal{G}(x')} - 1 \right]$, with $\mathcal{G}(x) = \frac{f^{-1} + x^n}{1 + x^n}$

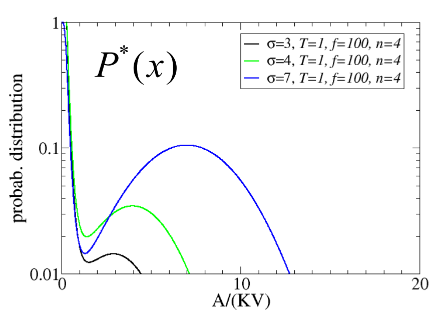


$$P^*(x) \propto \frac{1}{\mathcal{G}(x)} \exp\left\{-U(x)/T_{\text{eff}}\right\}$$

$$T_{\text{eff}} = (1+b)/(KV)$$

$$\text{for } b \sim 10,\ T_{\text{eff}} \approx \begin{cases} 0.1 & \text{for KV=100} \\ 1 & \text{for KV=10} \end{cases}$$





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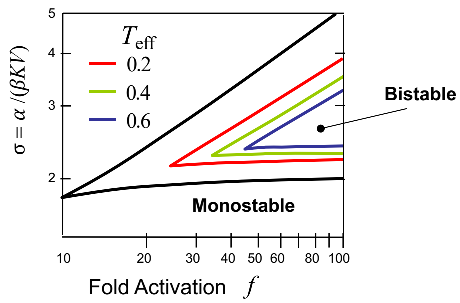
Summary on Langevin approach:

- effective thermodynamic formulation for nonequilibrium systems
- intuitive; qualitative effect of noise readily revealed
- kinetics of transition between stable states can be studied
- difficult to generalize to multiple variables

Approximation 3: perturbative expansion (in 1/N)

- van Kampen, *Adv. Chem. Phys.* **34**: 245 (1976)
- Scott et al, *PNAS* **104**: 7402 (2007)

➔ noise-corrected phase diagram
➔ regime of bistability significantly reduced



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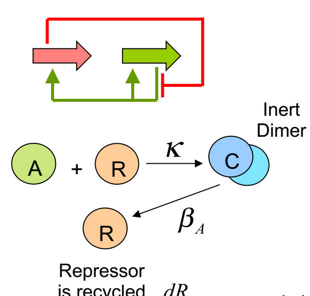
Effect of fluctuations on oscillatory circuits

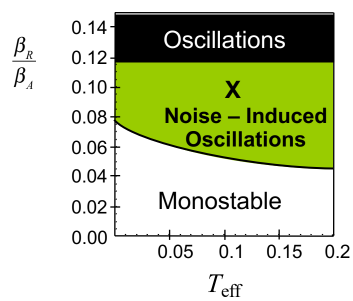
Mechanisms of noise-resistance in genetic oscillators
José M. G. Vilar*†, Hao Yuan Kueh*, Naama Barkai‡, and Stanislas Leibler*†§
5988–5992 | PNAS | April 30, 2002 | vol. 99

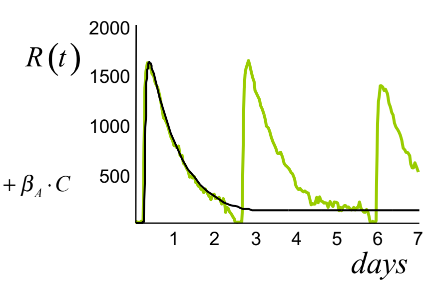


$$\frac{dR}{dt} = \alpha_R \cdot g_A(A) - \beta_R \cdot R - \kappa \cdot A \cdot R$$

$$\frac{dA}{dt} = \alpha_A \cdot g_A(A) - \beta_A \cdot A - \kappa \cdot A \cdot R + \beta_A \cdot C$$

$$\frac{dC}{dt} = \kappa \cdot A \cdot R - \beta_A \cdot C$$





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