Topic 4: Genetic Circuits

A. Models and behaviors of simple genetic circuits
B. Noise in gene expression
C. Metabolic control

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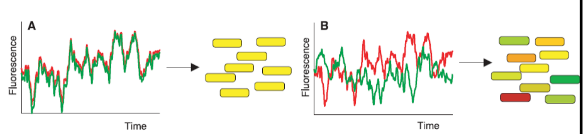
B. Genetic noise

- 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?

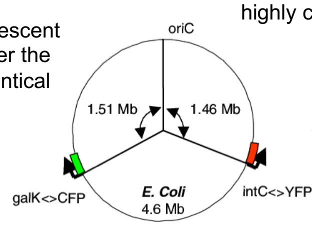
Stochastic Gene Expression in a Single Cell
Michael B. Elowitz,[1,2*] Arnold J. Levine,[1] Eric D. Siggia,[2] Peter S. Swain[2]
SCIENCE VOL 297 16 AUGUST 2002



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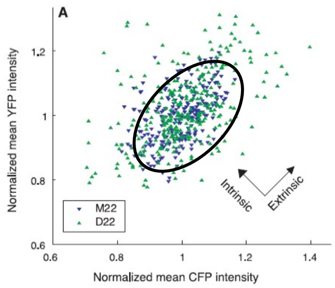
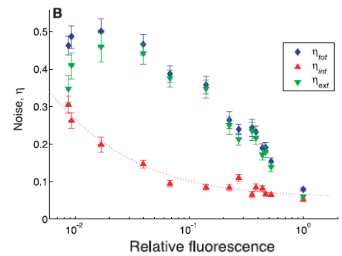
Scatter plot of mean fluorescence intensity/cell for many cells.



Population average at different inducer concentrations



➔ extrinsic noise larger, but intrinsic noise not negligible

➔ noise decreases with expression levels

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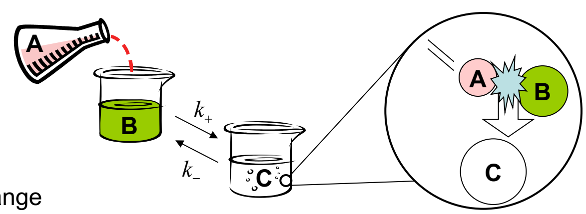
Intrinsic stochasticity

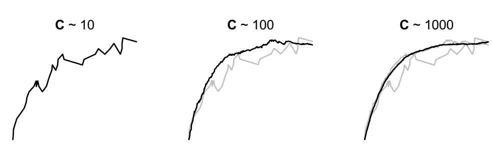
- rate equation:

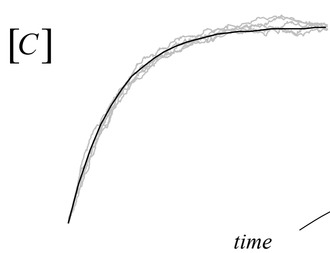
$$\frac{d[C]}{dt} = k_+[A][B] - k_-[C]$$

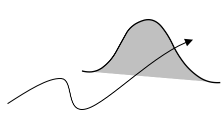
Continuous, differential change in the concentration of **C**

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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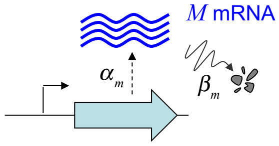
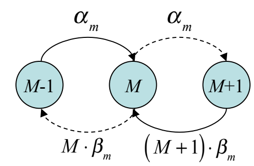
Consider mRNA synthesis



two reactions:

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



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

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

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

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

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

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

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

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

➔ avg = var signature of Poisson

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

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

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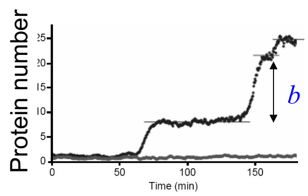
Next include translation

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

$$\approx 1 + \frac{\alpha_p}{\beta_m}$$ 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.]

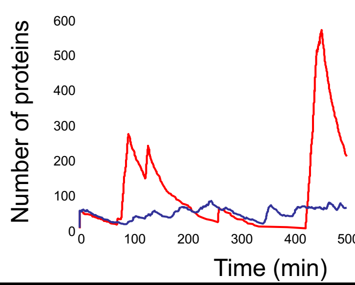
- Use the transition rate to compute a probability distribution for *when the next reaction will be completed*
- Use the transition rates to compute a probability distribution for *which reaction will occur*
- Update the state for each species of reactant

➔ 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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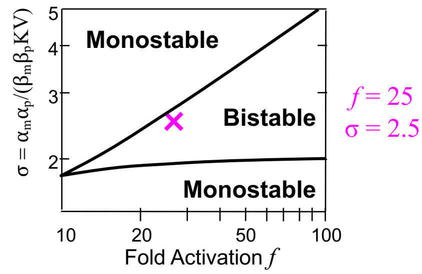
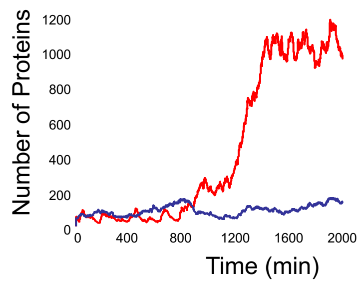
Apply to the autoactivator model

rate equations:

$$\dot{M} = \alpha_m \cdot \mathcal{G}(A/KV) - \beta_m \cdot M$$
$$\dot{A} = \alpha_p \cdot M - \beta_p \cdot A$$
$$\text{with } \mathcal{G} = \frac{f^{-1} + (A/KV)^n}{1 + (A/KV)^n}.$$

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

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

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

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

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

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

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

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

- 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 $P^*(A)$

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

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

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

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

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

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

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

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

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

Probability being in the high state reduced f-fold

effective potential $U(A/KV)$

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 $\mathcal{G}(x) = \frac{f^{-1} + x^n}{1 + x^n}$

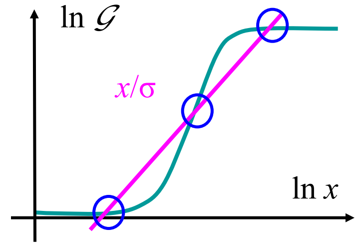
$$\frac{dU}{dx} = \frac{x}{\sigma \cdot \mathcal{G}(x)} - 1 \xrightarrow{\left.\frac{dU}{dx}\right|_{x^*} = 0} \sigma \cdot \mathcal{G}\left(x^*\right) = x^*$$

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

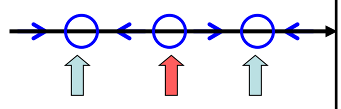
➔ extrema of U(x) ⇔ fixed points

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

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



stability analysis (graphical):



➔ robustness of bistability to stochastic fluctuations: compare "barrier height" to effective temperature

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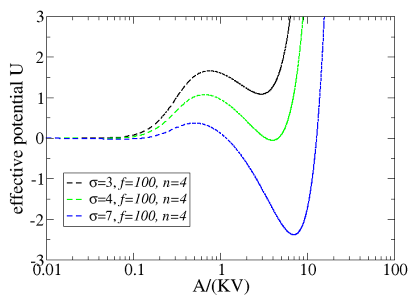
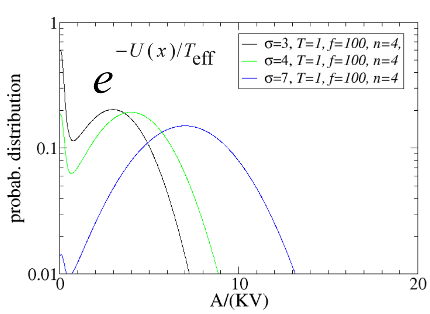
Effective potential $U(x) = \int^x dx' \left[\frac{x'}{\sigma \cdot \mathcal{G}(x')} - 1 \right]$, with $\mathcal{G}(x) = \frac{f^{-1} + x^n}{1 + x^n}$

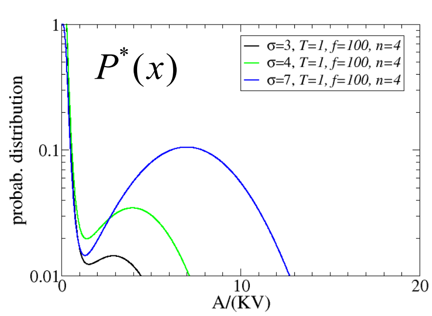


$$P^*(x) \propto \frac{1}{\mathcal{G}(x)} \exp\left\{-U(x)/T_{\text{eff}}\right\}$$

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

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





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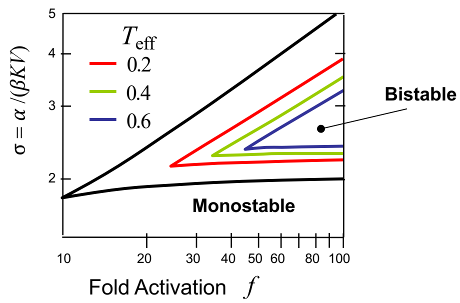
Summary on Langevin approach:

- 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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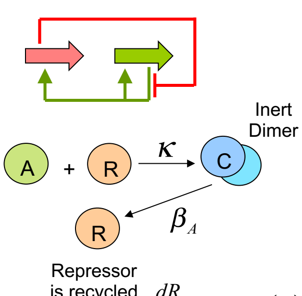
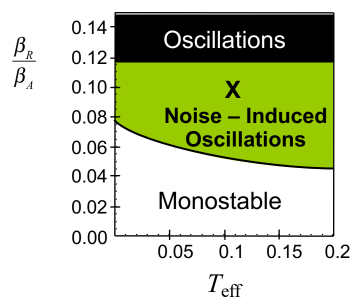
Effect of fluctuations on oscillatory circuits

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

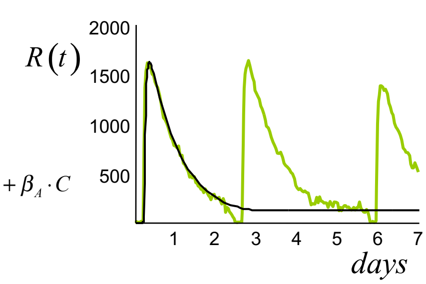




$$\frac{dR}{dt} = \alpha_R \cdot g_A(A) - \beta_R \cdot R - \kappa \cdot A \cdot R$$

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

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



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