Stochastic modeling of gene regulatory networks

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MICROCOSME: bacterial systems biology

- **MICROCOSME**: systems biology group at INRIA/Université Grenoble Alpes in Grenoble

  Microbiologists, computer scientists, mathematicians, physicists, …

https://team.inria.fr/microcosme

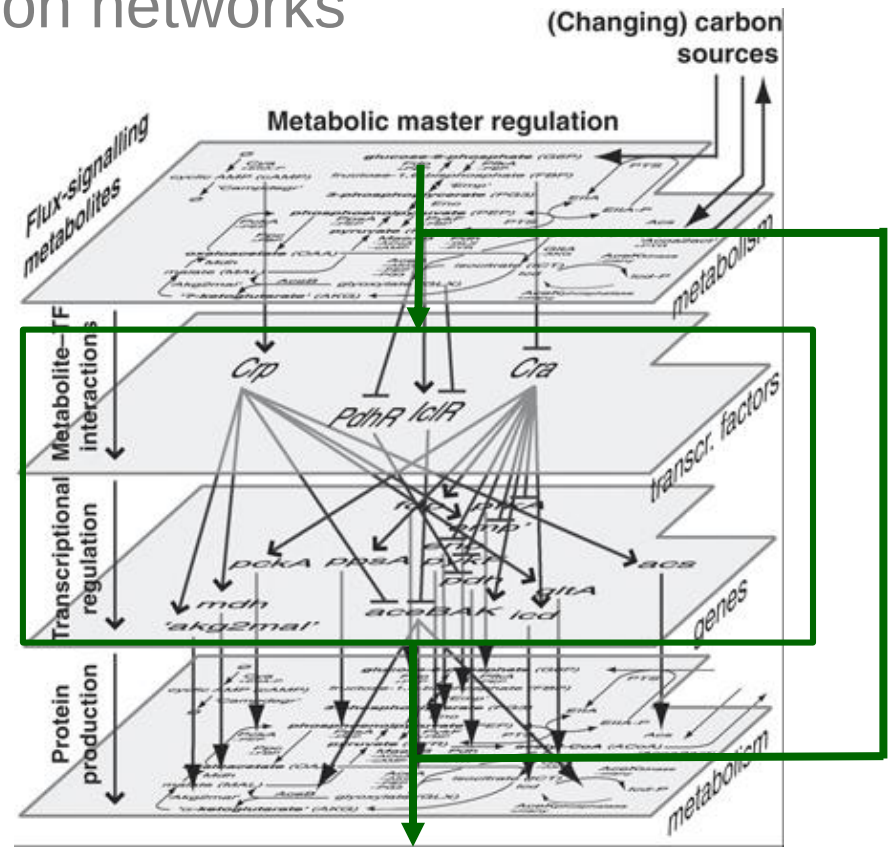
- **Objective:** analysis, engineering, and control of the growth of bacteria
  - Specific research problems shaped by **biological questions**
  - Problems often addressed by combination of **models and experiments**

Overview

- Part 1. Systems biology and kinetic modeling
- Part 2. Metabolic network modeling
- Part 3. Gene regulatory network modeling
  - Quantitative modeling of gene regulatory networks
  - Qualitative modeling of gene regulatory networks
  - **Stochastic modeling of gene regulatory networks**
  - Practical on integrated models of bacterial growth (Matlab)

Gene regulatory networks

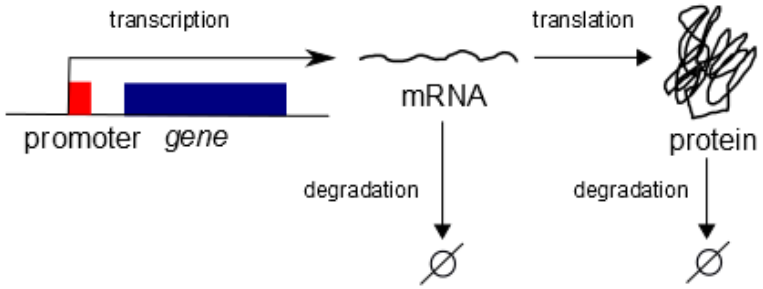
- Focus on **subsystems** that can be studied in isolation due to **modular structure** of reaction networks
  - Time-scale hierarchies
  - Connectivity structure
- **Gene regulatory networks**
  - Genes, proteins, and regulatory interactions
  - Reactions involved in transcription and translation and their regulation
  - Time-scale: min (mRNA) to h (proteins)



Kotte *et al.* (2010), *Mol. Syst. Biol.*, 6: 355

Gene expression

- Typically, and simplifying quite a bit, **gene expression** in bacteria involves:
  - Transcription by RNAP (mRNA)
  - Translation by ribosomes (proteins)
  - Degradation of mRNA and protein

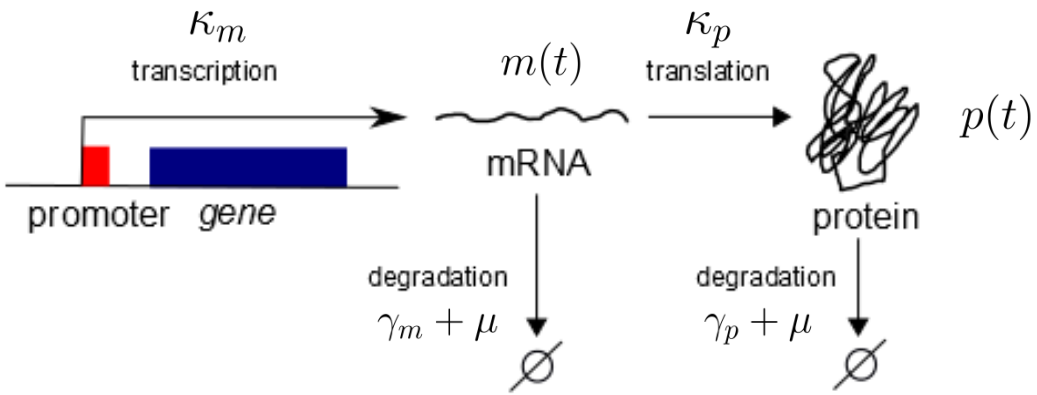


Modeling of gene regulatory networks

- ODE model of gene expression, distinguishing **transcription** and **translation**

$$\dot{m} = \kappa_m - (\gamma_m + \mu)\, m$$

$$\dot{p} = \kappa_p\, m - (\gamma_p + \mu)\, p$$



$m(t) \geq 0$, concentration mRNA

$p(t) \geq 0$, concentration protein

$\kappa_m, \kappa_p > 0$, synthesis rate constants

$\gamma_m, \gamma_p > 0$, degradation rate constants

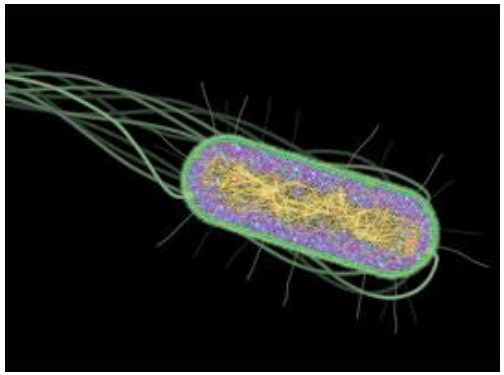
$\mu \geq 0$, growth rate

Stochasticity in gene expression

- ODE models make abstraction of underlying biochemical reaction processes involved in gene expression that may not be warranted

Kaern *et al.* (2005), *Nat. Rev. Genet.*, 6(6):451-464

- Gene expression is **stochastic** instead of **deterministic** process
  - Underlying biochemical reactions are stochastic processes
  - Probability of reaction to occur depends on random encounters of molecules in cell



Goodsell (2010), *The Machinery of Life, Springer,* 2nd ed.

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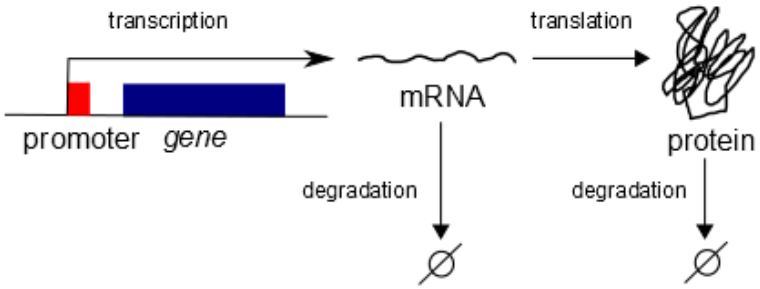
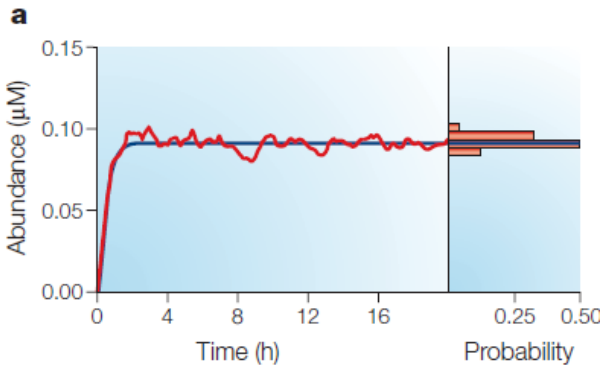
- **Discrete** number of molecules of reaction species, instead of **continuous** concentrations

  Some reactions species involved in gene expression have very low copy numbers (1-10)

Stochasticity in gene expression

- Stochasticity in gene expression leads to **noise**
  - Fluctuations in mRNA and protein concentrations





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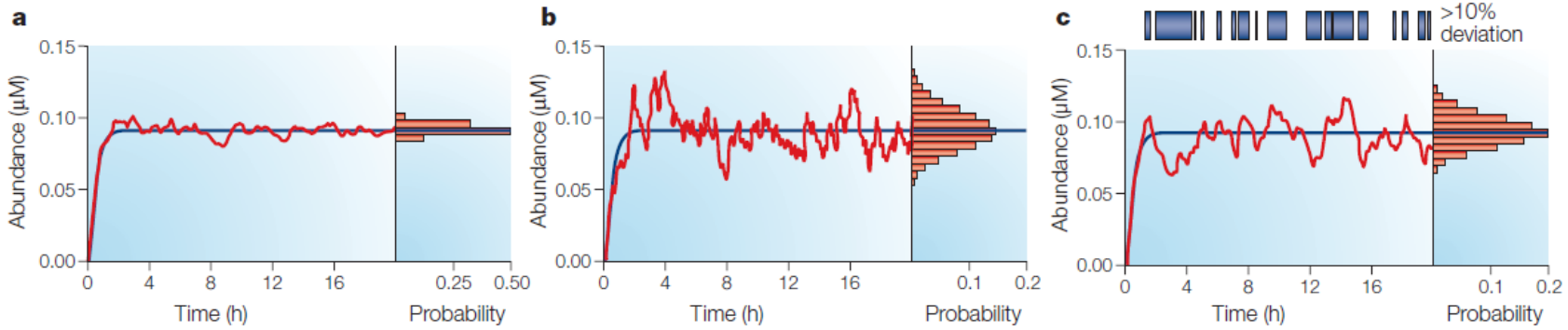
Stochasticity in gene expression

- Stochasticity in gene expression leads to **noise**
  - Fluctuations in mRNA and protein concentrations
- Noise amplified by small number of molecules



Decrease in number of mRNA and protein molecules wrt **a**

Decrease in number of mRNA molecules wrt **a**, for same number of protein molecules

Kaern *et al.* (2005), *Nat. Rev. Genet.*, 6(6):451-464

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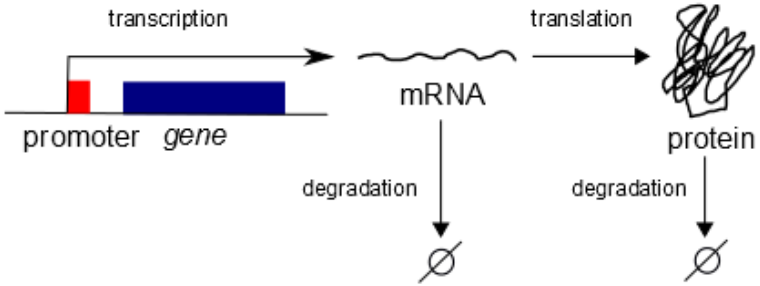

- Different types of noise:
  - **Intrinsic noise:** fluctutations due to stochasticity of processes involved in gene expression (transcription, translation, …)

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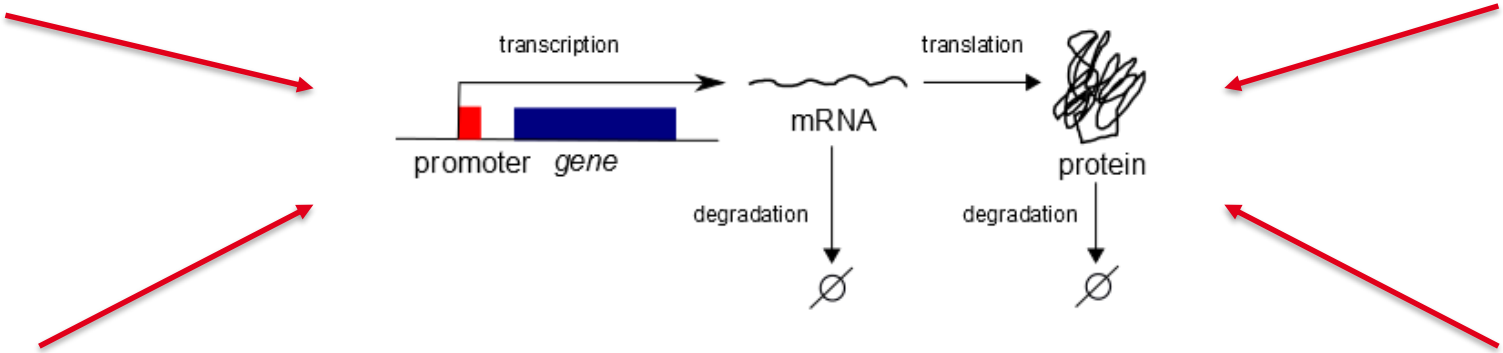
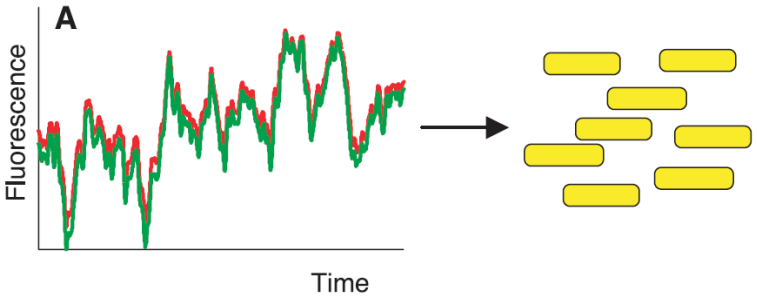


- Different types of noise:
  - **Intrinsic noise:** fluctutations due to stochasticity of processes involved in gene expression (transcription, translation, …)
  - **Extrinsic noise:** fluctuations due to variability in external factors (temperature, ribosome availability, …). Impact on rate constants.

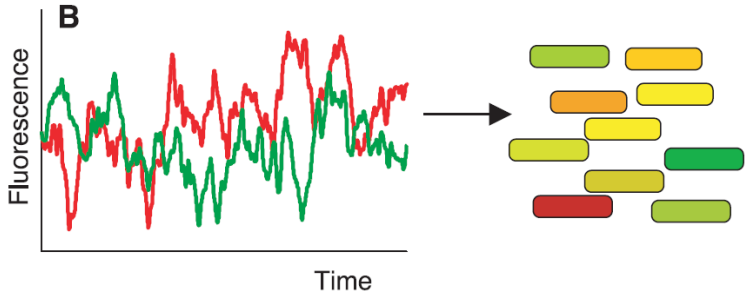
Stochasticity in gene expression

- Experimental discrimination between intrinsic and extrinsic noise

  Expression in a single cell with two different reporter genes (*gfp* and *cfp*) controlled by same promoter



No intrinsic noise, so relative amount of both proteins is constant over time and across individual cells in population



Intrinsic noise, so relative amount of both proteins varies over time and across individual cells in population

Elowitz *et al.* (2002), *Science*, 297(5584):1183-6

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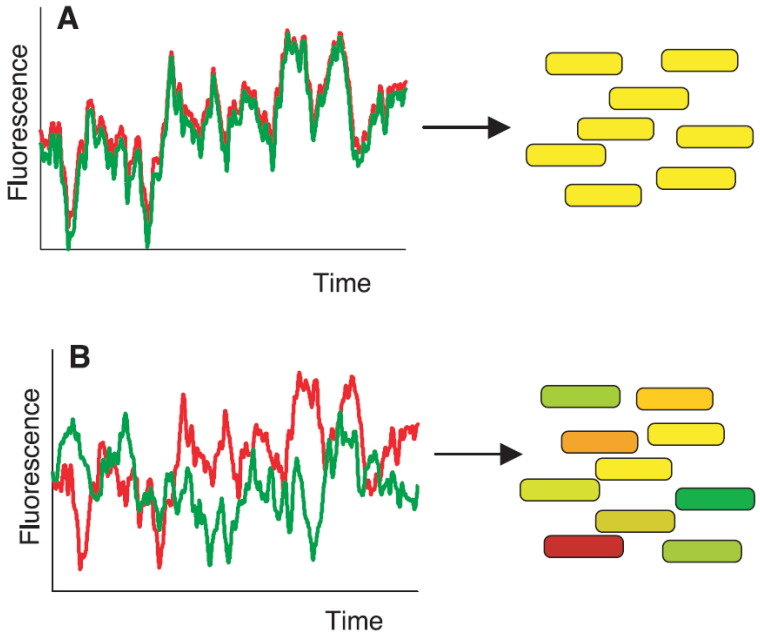

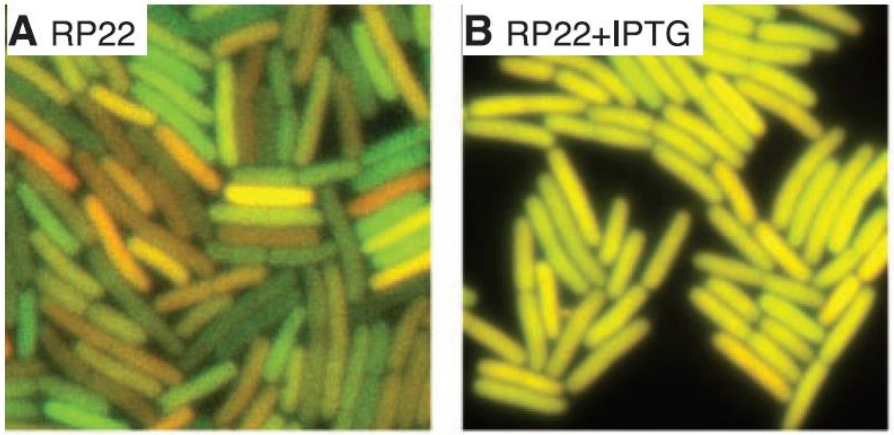

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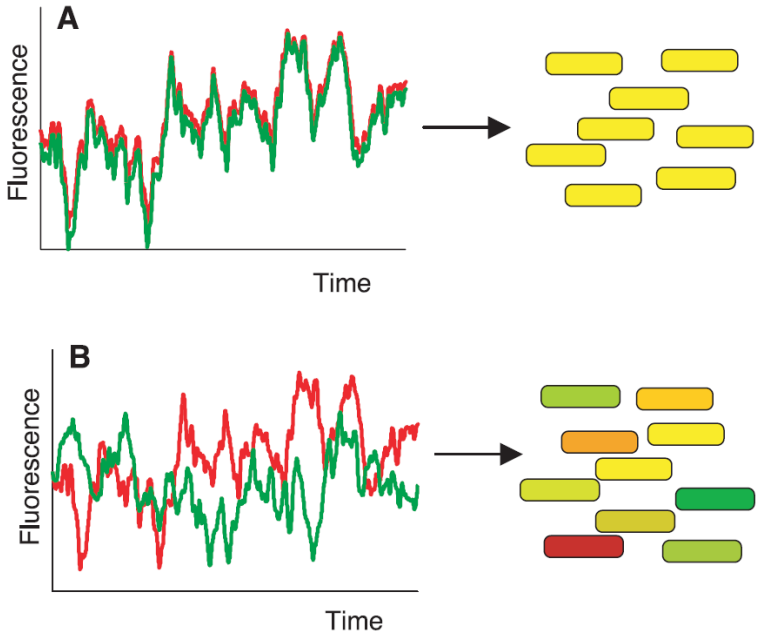
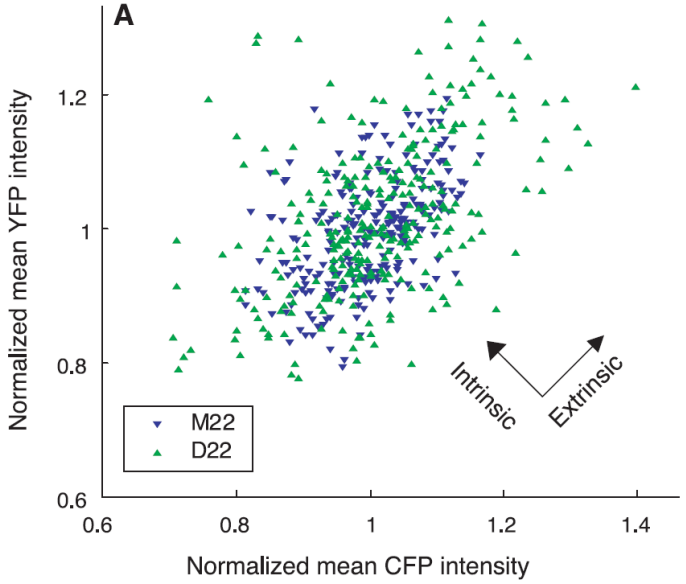




Elowitz *et al.* (2002), *Science*, 297(5584):1183-6

Stochasticity in gene expression

- Major question is how cells both **tolerate and exploit** noise.

  Rao *et al.* (2002), *Nature*, 420(6912):231-237

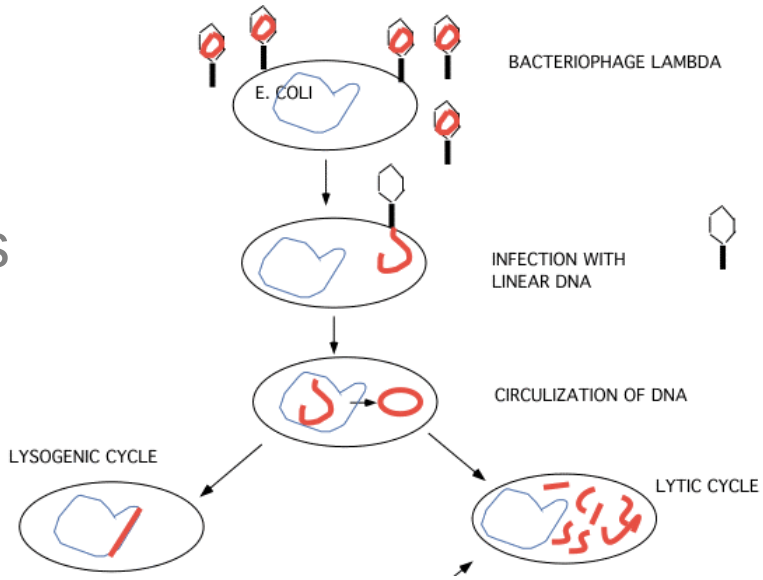
  Raj and van Oudenaarden (2008), *Cell*, 135(2):216-26

- Most cellular processes are **robust** to noise, despite stochasticity of underlying system of biochemical reactions

- Sometimes, intracellular noise drives **population heterogeneity** that may be beneficial for a species

  After infection, only fraction of cells lyse

- ODE models are not suitable for studying origin and effects of noise



Stochastic models

- **Stochastic models** of gene regulation are more appropriate
- Number of molecules of each species i at time-point t represented by discrete variable $X_i(t) \in \mathbb{N}$

X_1 X_2 X_3 X_4

Stochastic models

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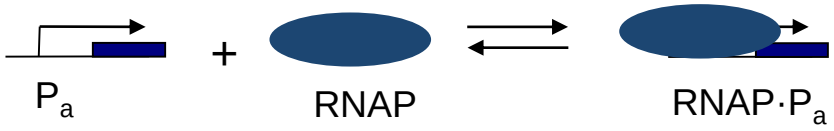
- Number of molecules of each species i at time-point t represented by discrete variable $X_i(t) \in \mathbb{N}$
- Reactions between molecular species lead to change in state of system from $\boldsymbol{X}(t)$ to $\boldsymbol{X}(t+\Delta t)$ over time-interval Δt, where $\boldsymbol{X} = [X_1, \ldots, X_n]'$

  Change of state by reaction k described by vector $\boldsymbol{\nu}_k$

Reaction 1: $\boldsymbol{\nu}_1 = [-1\ -1\ 1\ 0]$



Reaction 2: $\boldsymbol{\nu}_2 = [1\ 1\ -1\ 1]$

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$X_1 = 1$ $X_2 = 7$ $X_3 = 0$ $X_4 = 10$

Reaction 1: ν_1 = [-1 -1 1 0]

$X_1 = 0$ $X_2 = 6$ $X_3 = 1$ $X_4 = 10$

Stochastic models

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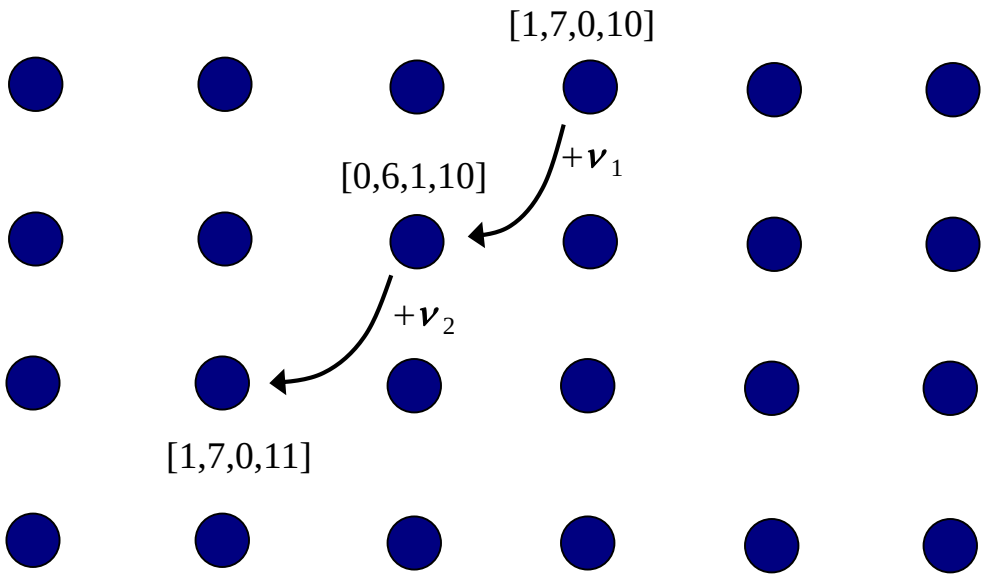
$X_1 = 0$ $X_2 = 6$ $X_3 = 1$ $X_4 = 10$

Reaction 2: ν_2 = [1 1 -1 1]

$X_1 = 1$ $X_2 = 7$ $X_3 = 0$ $X_4 = 11$

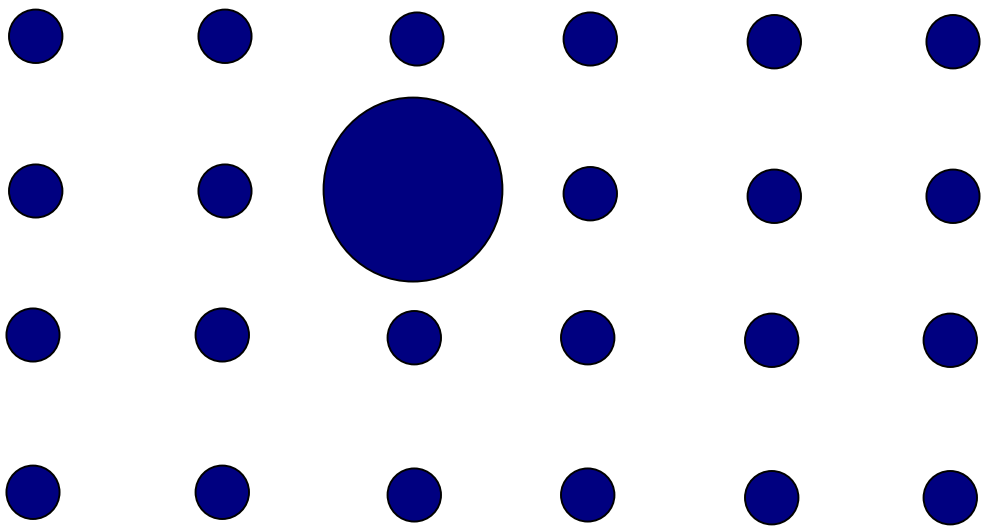
Stochastic models

- Possible states are given by possible value combinations for variables: $\boldsymbol{X} = \boldsymbol{V}$, with $\boldsymbol{V} = [V_1, \ldots, V_n]'$
- Transitions between states are given by possible reactions k



Stochastic models

- Probability distribution $\mathrm{p}[\boldsymbol{X}(t)=\boldsymbol{V}]$ describes probability that at time-point t there are $\boldsymbol{V}=[V_1,\ldots,V_n]'$ molecules



Time t_0

Stochastic models

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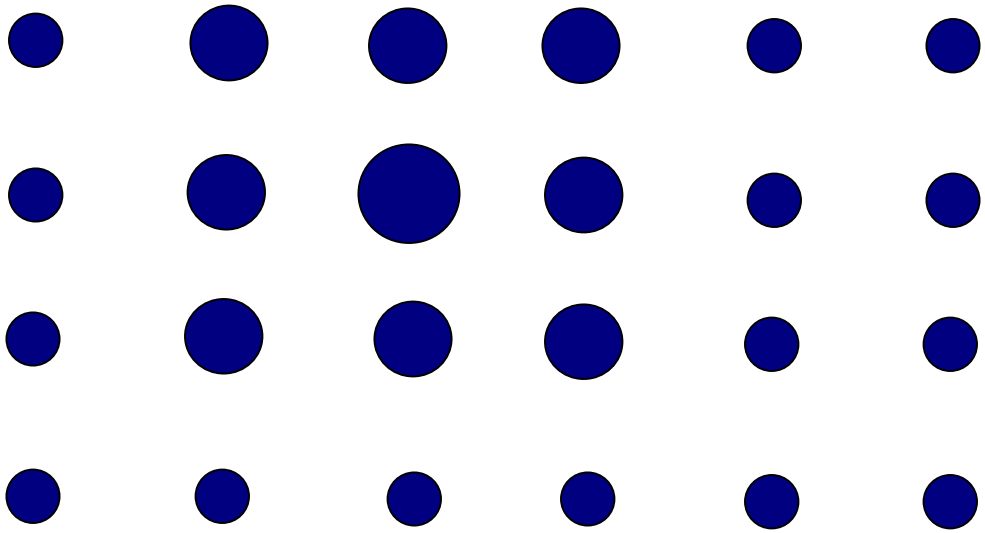

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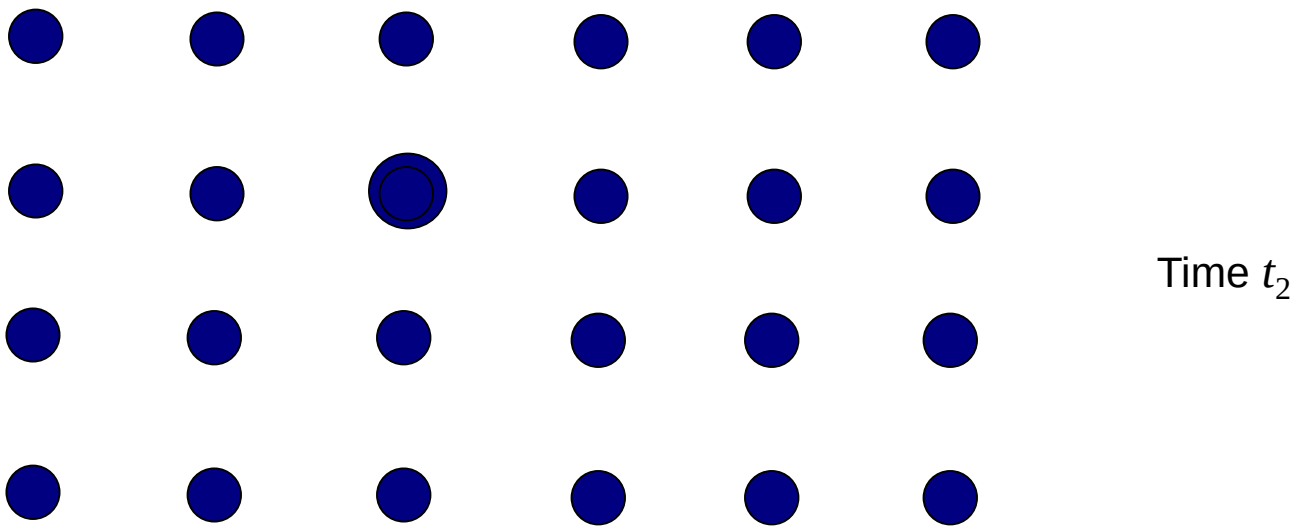

Time t_2

Stochastic master equation

- Evolution of probability distribution $p[\boldsymbol{X}(t)=\boldsymbol{V}]$ given by

$$p[\boldsymbol{X}(t+\Delta t)=\boldsymbol{V}] = p[\boldsymbol{X}(t)=\boldsymbol{V}](1-\sum_{j=1}^{m}\alpha_j \Delta t) + \sum_{k=1}^{m} p[\boldsymbol{X}(t)=\boldsymbol{V}-\boldsymbol{\nu}_k]\,\beta_k \Delta t$$

  - m is the number of reactions that can occur in the system
  - $\alpha_j \Delta t$ is the probability that reaction j will occur in $[t, t+\Delta t]$ given that $\boldsymbol{X}(t)=\boldsymbol{V}$
  - $\beta_k \Delta t$ is the probability that reaction k will bring the system from $\boldsymbol{X}(t)=\boldsymbol{V}-\boldsymbol{\nu}_k$ to $\boldsymbol{X}(t+\Delta t)=\boldsymbol{V}$ in $[t, t+\Delta t]$

Van Kampen (1997), *Stochastic Processes in Physics and Chemistry*, Elsevier

Stochastic master equation

- For $\Delta t \to 0$ we obtain **stochastic master equation**

$$\mathrm{d}p[\boldsymbol{X}(t)=\boldsymbol{V}] / \mathrm{d}t = \sum_{j=1}^{m} p[\boldsymbol{X}(t)=\boldsymbol{V}-\boldsymbol{\nu}_j]\ \beta_j - p[\boldsymbol{X}(t)=\boldsymbol{V}]\ \alpha_j$$

- Probabilities α_j, β_j are defined in terms of kinetic constants of reactions and number of reactant molecules
- Unimolecular reaction j : $S_1 \rightarrow$ product(s)

$$\alpha_j = k_j\, X_1\, (X_1\text{-}1)/2$$

- Bimolecular reaction j : $S_1 + S_2 \rightarrow$ product(s)

$$\alpha_j = k_j\, X_1\, X_2/\Omega$$

Ω : cell volume

Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

Stochastic master equation

- For $\Delta t \to 0$ we obtain **stochastic master equation**

$$\mathrm{d}\mathrm{p}[\boldsymbol{X}(t)=\boldsymbol{V}] / \mathrm{d}t = \sum_{j=1}^{m} \mathrm{p}[\boldsymbol{X}(t)=\boldsymbol{V}-\boldsymbol{\nu}_j]\ \beta_j - \mathrm{p}[\boldsymbol{X}(t)=\boldsymbol{V}]\ \alpha_j$$

- Probabilities α_j, β_j are defined in terms of kinetic constants of reactions and number of reactant molecules
- Analytical solution of master equation is not possible in most situations of practical interest

Van Kampen (1997), *Stochastic Processes in Physics and Chemistry*, Elsevier

Stochastic simulation

- Analytical solution of master equations is not possible in most situations of practical interest
- **Stochastic simulation** generates sequences of reactions that change state of system, starting from initial state $\boldsymbol{X}(0) = \boldsymbol{V_0}$
  - Stochastic simulation samples joint probability density function

    $$\mathrm{p}[\tau, j|\boldsymbol{X}(t) = \boldsymbol{V}]$$

    τ = time until occurrence of next reaction

    j = index of next reaction
  - **Interpretation:** $\mathrm{p}[\tau, j|\boldsymbol{X}(t) = \boldsymbol{V}]\mathrm{d}\tau$ is probability, given $\boldsymbol{X}(t) = \boldsymbol{V}$, that next reaction will occur in $[t+\tau, t+\tau+\mathrm{d}\tau]$ and is reaction j

Gillespie (2002), *J. Phys. Chem.*, 81(25): 2340-61

Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

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    τ = time until occurrence of next reaction

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  - Probability density function defined in terms of α_j, β_k (reaction constants)

Gillespie (2002), *J. Phys. Chem.*, 81(25): 2340-61

Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

Stochastic simulation

- Analytical solution of master equations is not possible in most situations of practical interest
- **Stochastic simulation** generates sequences of reactions that change state of system, starting from initial state $\boldsymbol{X}(0) = \boldsymbol{V_0}$
- Stochastic simulation based on sampling of $\mathrm{p}[\tau, j|\boldsymbol{X}(t) = \boldsymbol{V}]$ generates sequences in exact accordance with stochastic master equations
- Repeating stochastic simulation many times (Monte-Carlo procedure) yields approximation of probability distribution $\mathrm{p}(\boldsymbol{X}(t)=\boldsymbol{V})$

Gillespie (2002), *J. Phys. Chem.*, 81(25): 2340-61

Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

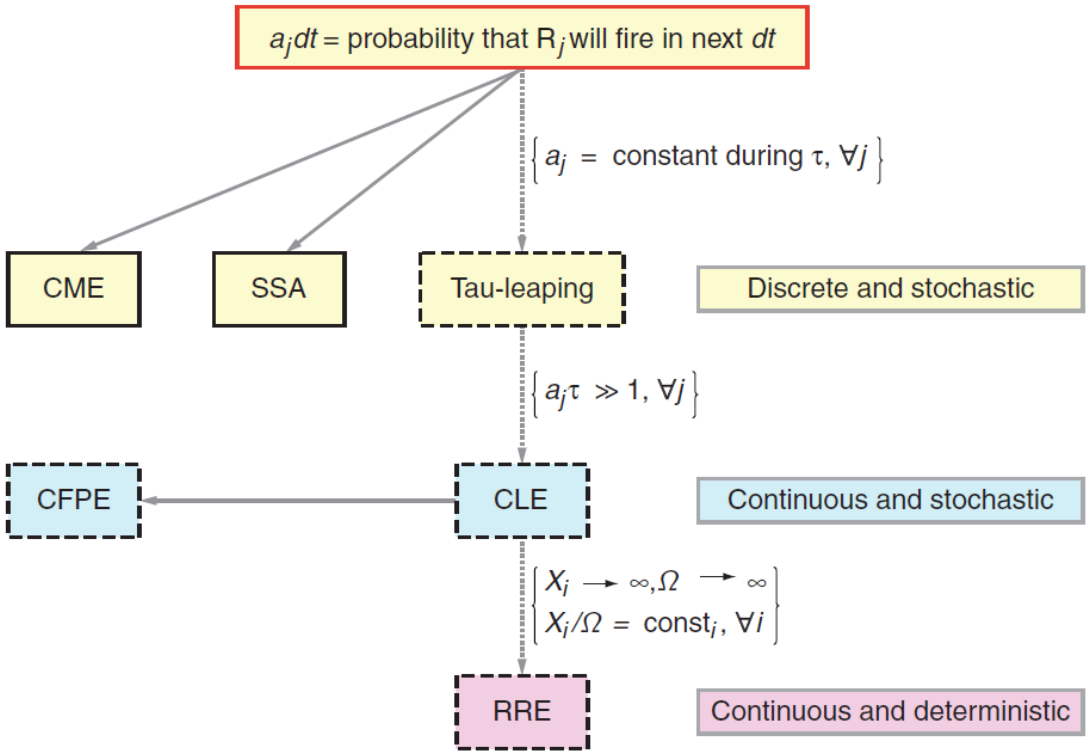
Stochastic simulation

- Analytical solution of master equations is not possible in most situations of practical interest
- **Stochastic simulation** generates sequences of reactions that change state of system, starting from initial state $\boldsymbol{X}(0) = \boldsymbol{V_0}$
- Various approximations of basic stochastic simulation algorithm, trading exactness for simulation speed:
  - Tau-leaping approaches: choose τ such that α_j, β_j remain approximately constant over time interval (encapsulate several reactions in one step)
  - Quasi-steady-state approximations (distinguish between slow and fast reactions)
  - …

Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

Stochastic simulation

- Relation of stochastic simulation models with other modeling approaches



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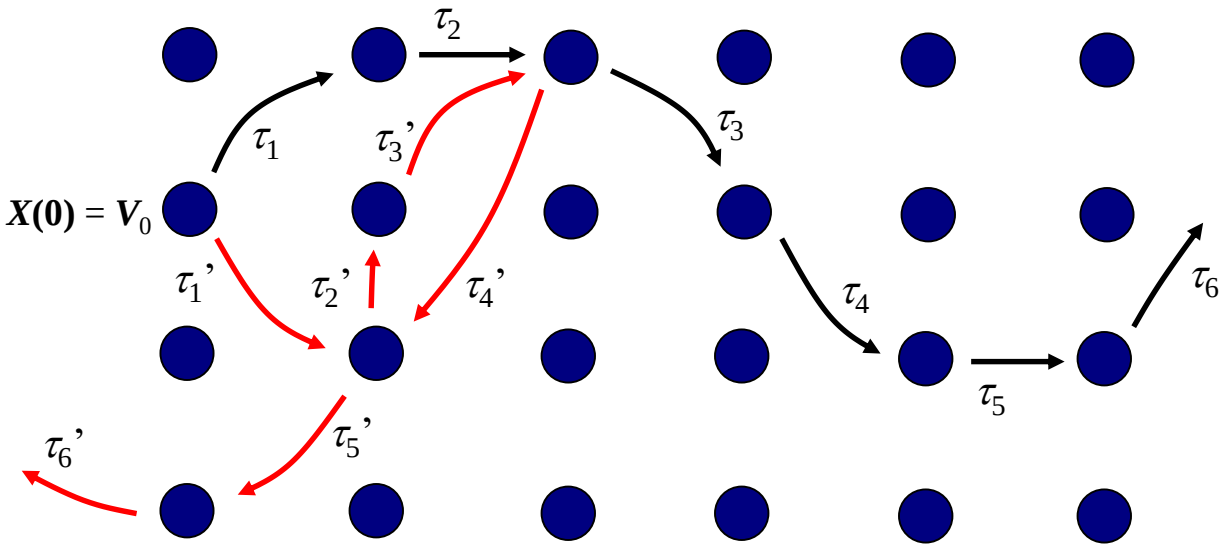
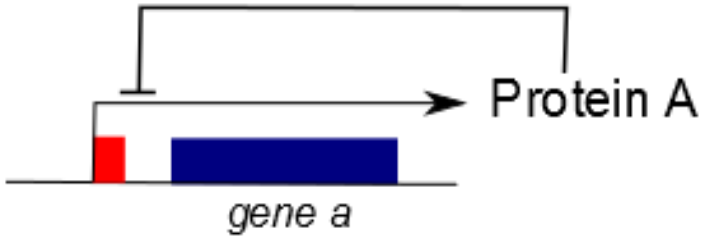


- Stochastic simulation may lead to different dynamical behaviors starting from identical initial conditions: **heterogeneity**

Auto-inhibition network

- **Auto-inhibition** network consists of a single gene, coding for transcription regulator inhibiting expression of its own gene

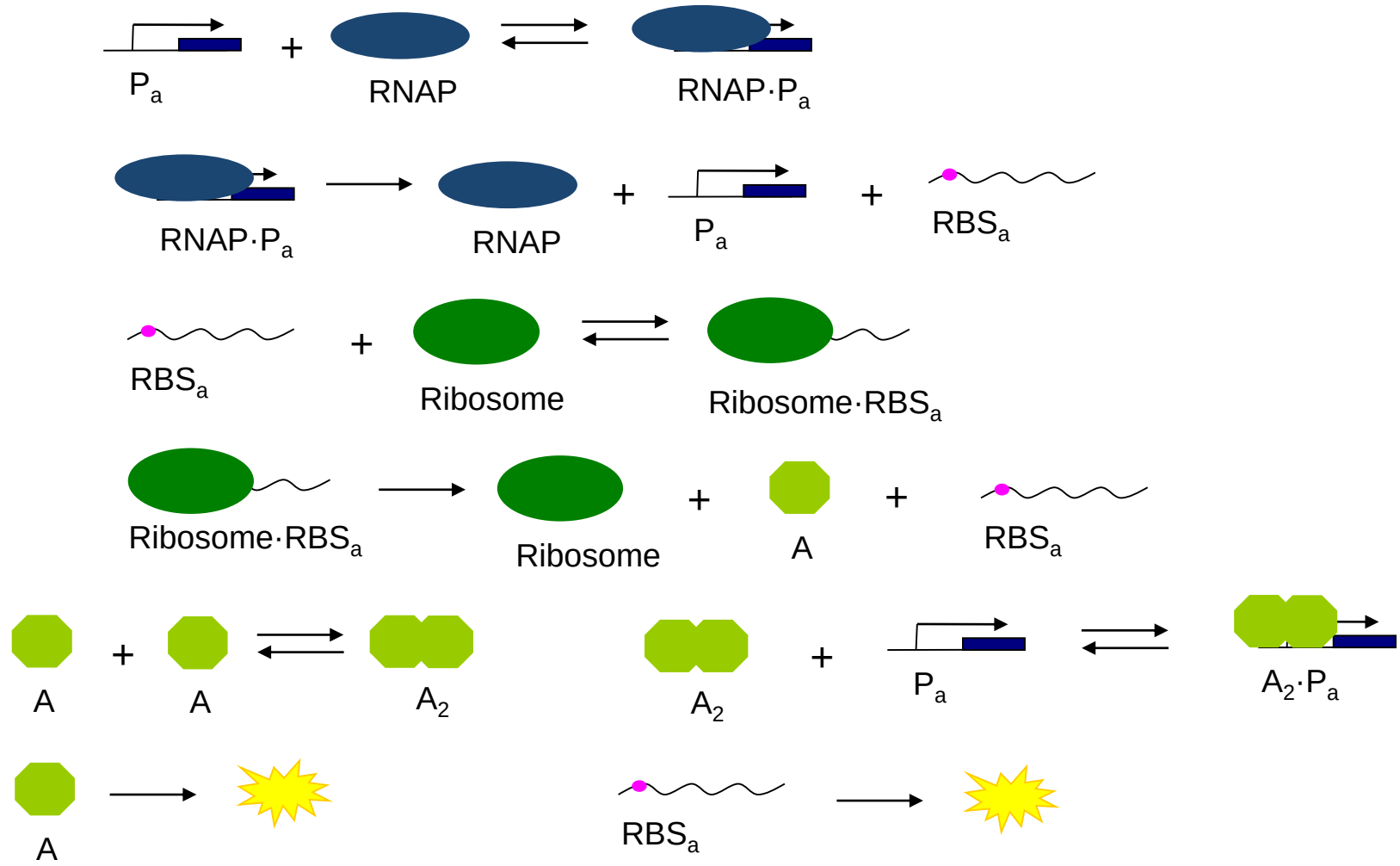


- Auto-inhibition is example of **negative feedback**, and frequently occurs in bacterial regulatory networks

Thieffry *et al*. (1998), *BioEssays*, 20(5):433-440

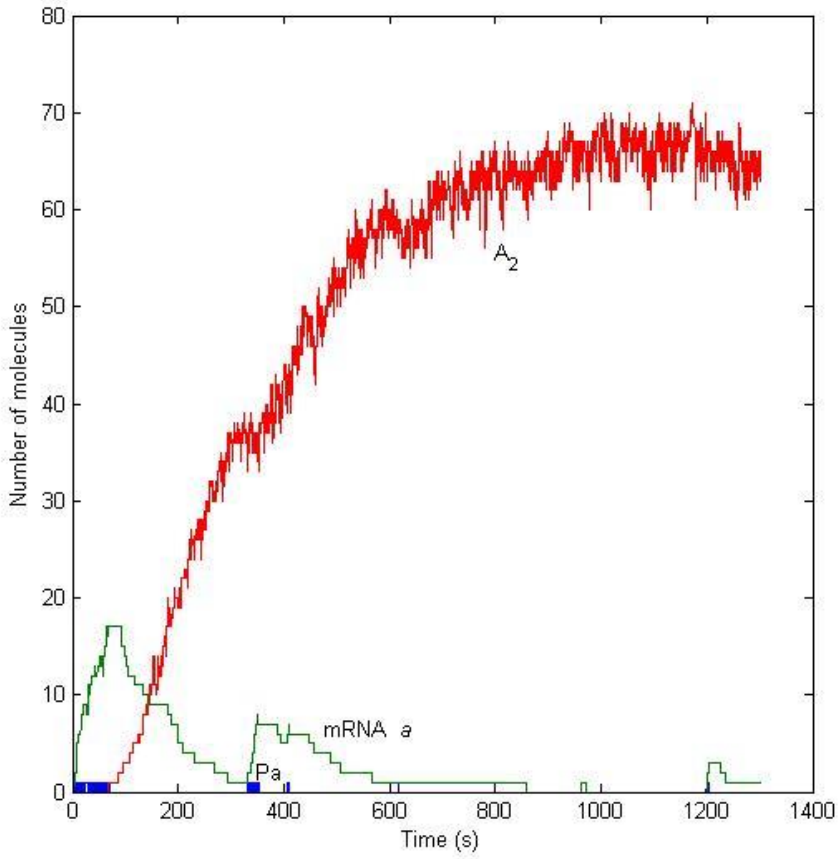
- Development of stochastic model requires list of species, reactions, and kinetic constants

Reactions and species



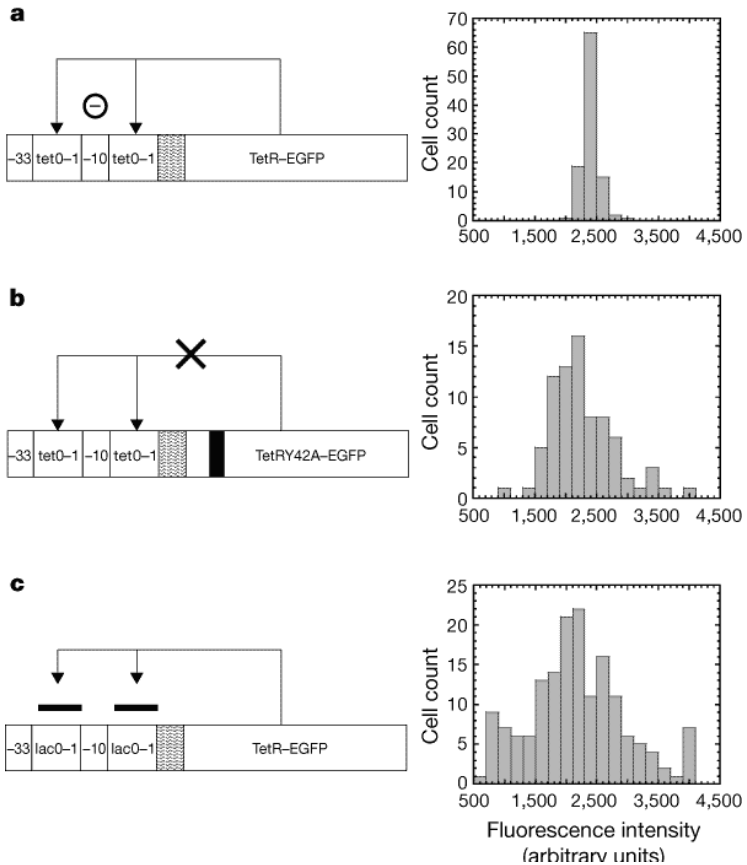
Stochastic simulation of auto-inhibition

- Occurrence of fluctuations and bursts in gene expression



Auto-inhibition and noise reduction

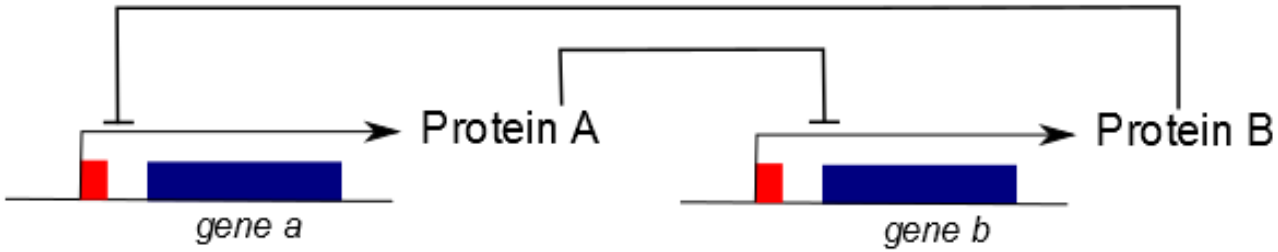
- Auto-inhibition reduces fluctuations in gene expression level



Becskei and Serrano (2000), *Nature*, 405(6785):590-591

Cross-inhibition network

- **Cross-inhibition** network consists of two genes, each coding for transcription regulator inhibiting expression of other gene



- Cross-inhibition network is example of **positive feedback**, important for phenotypic differentiation (multi-stability)

Thomas and d'Ari (1990), *Biological Feedback*, CRC Press

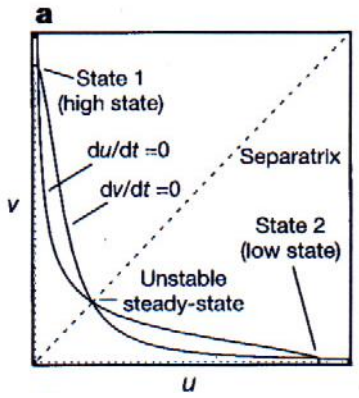
- Construction of cross inhibition network *in vivo:* **toggle switch**

Gardner *et al.* (2000), *Nature*, 403(6786): 339-42

Dynamics of toggle switch

- ODE model predicts bistability of toggle switch

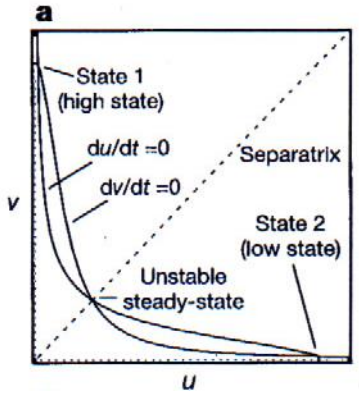
Gardner *et al.* (2000), *Nature*, 403(6786): 339-42

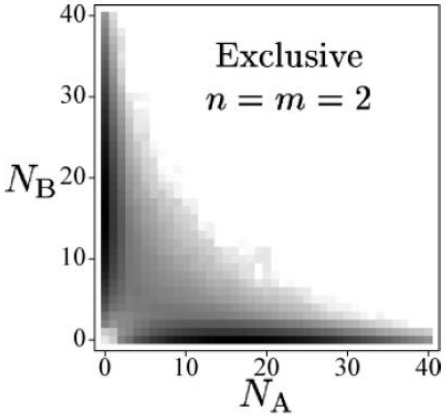


- Question: what will be predicted long-term dynamics in stochastic model of toggle switch?

Dynamics of toggle switch

- ODE model predicts bistability of toggle switch





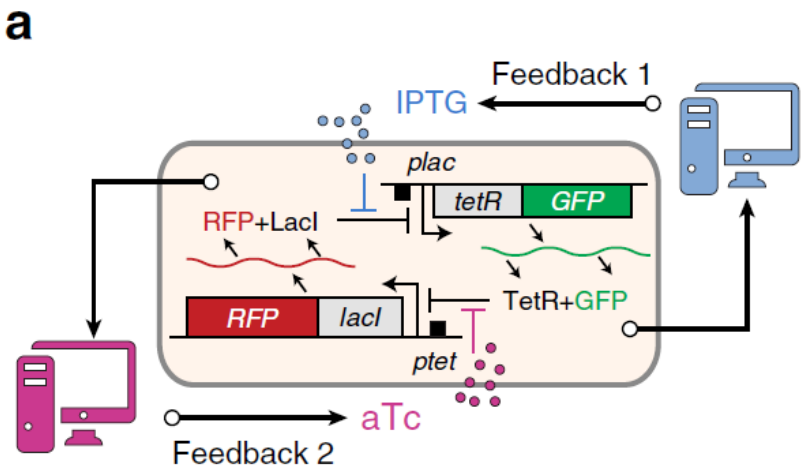
- Stochastic model predicts bimodal state (two attractors)

Warner and ter Wolde (2005), *J. Phys. Chem. B*, 109(4):6812-23

- Depending on noise characteristics, system can **spontaneously switch** from one attractor to another

Control of toggle switch

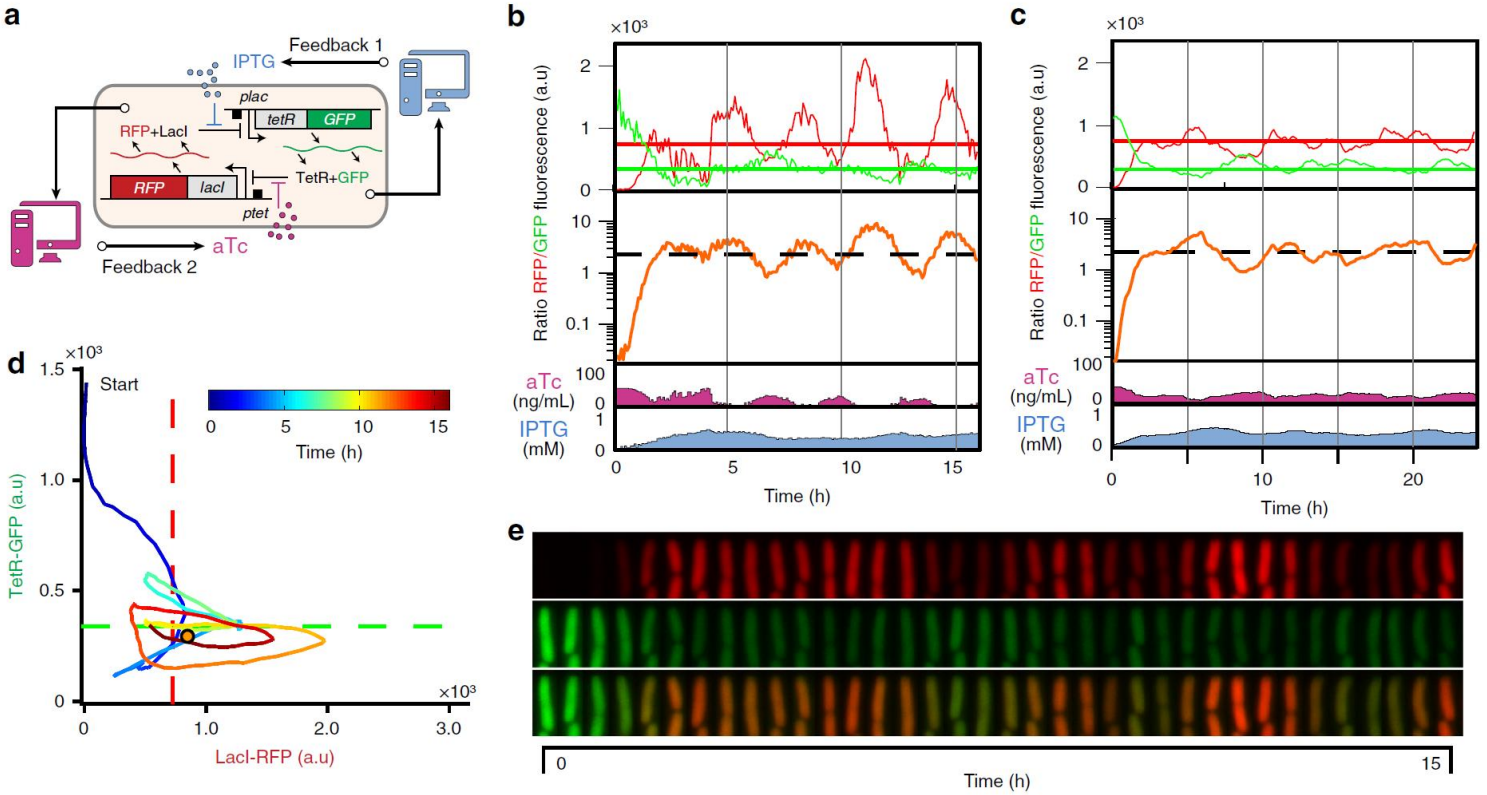
- Is it possible to stabilize toggle switch around unstable steady state in ODE model?



Lugagne *et al.* (2017), *Nat. Commun.*, 8:1671

Control of toggle switch

- Is it possible to stabilize toggle switch around unstable steady state in ODE model?



Lugagne *et al.* (2017), *Nat. Commun.*, 8:1671

Control of toggle switch

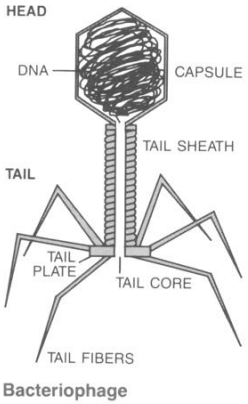
- Is it possible to stabilize toggle switch around unstable steady state in ODE model?
- Applications of control theory in synthetic biology: **cybergenetics**

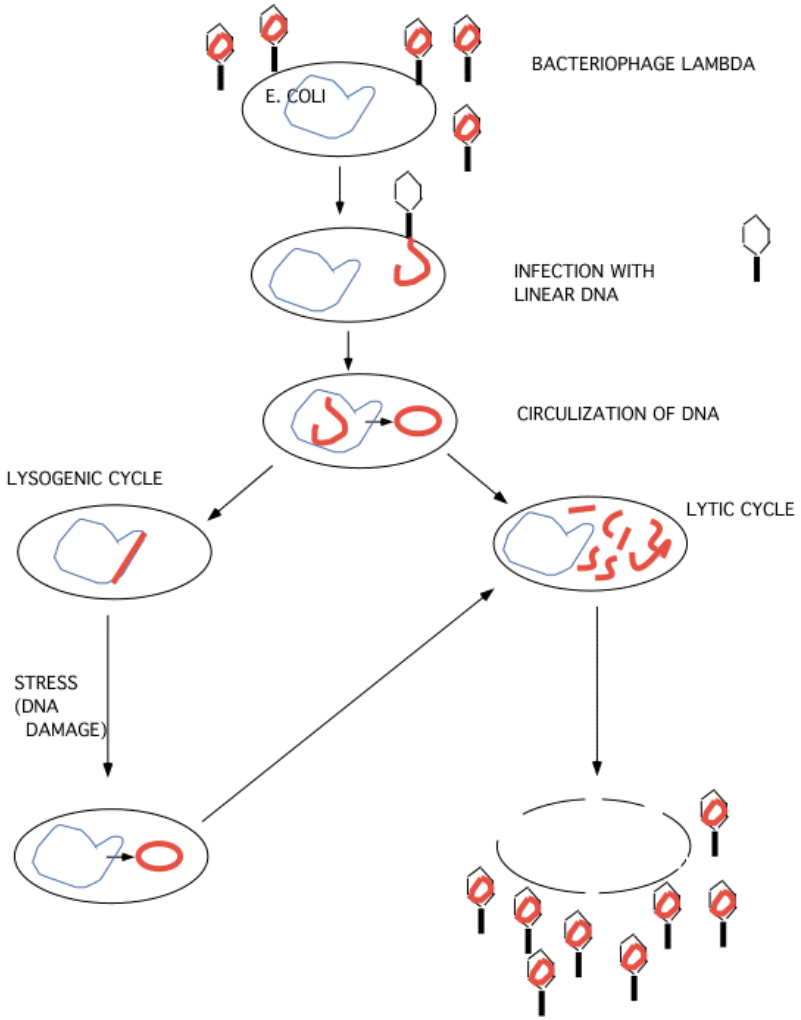
https://bsse.ethz.ch/ctsb/research/cybergenetics.html

Bacteriophage λ infection of *E. coli*

- Response of *E. coli* to phage λ infection involves decision between alternative developmental pathways: **lytic cycle** and **lysogeny**

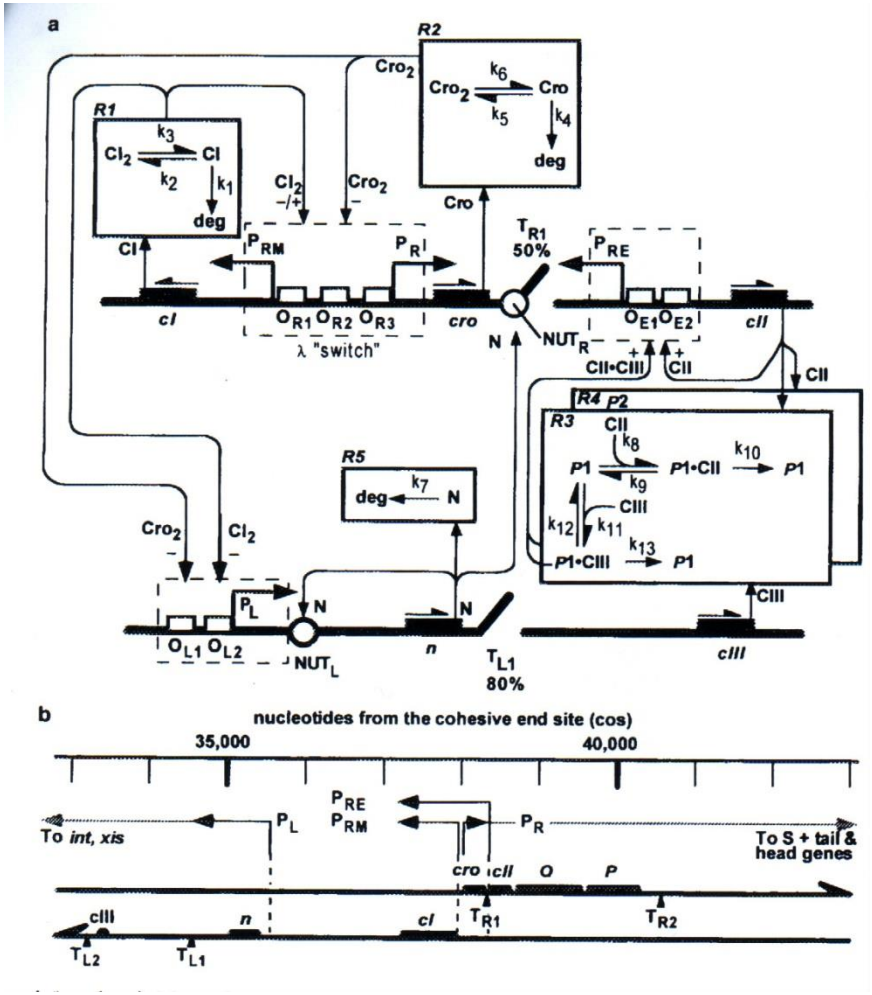
Ptashne (1997), *A Genetic Switch: Phage λ and Higher Organisms*, Cell Press





Stochastic analysis of phage λ infection

- Stochastic model of λ lysis-lysogeny decision network

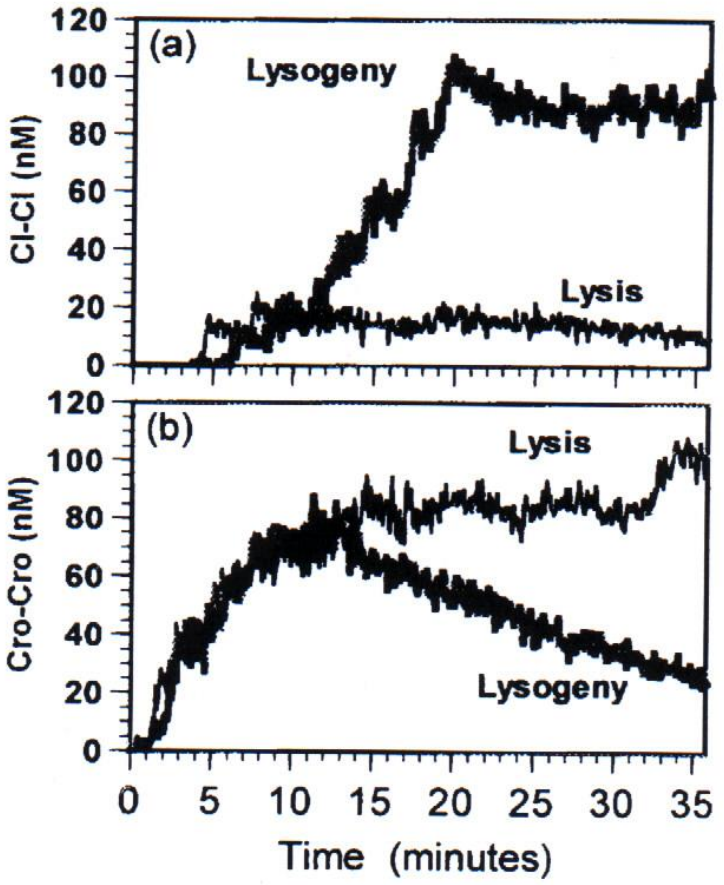


Arkin *et al.* (1998), *Genetics*, 149(4): 1633-1648

Stochastic analysis of phage λ infection

- Time evolution of Cro and CI dimer concentrations

- Due to stochastic fluctuations, under identical conditions cells follow one or other pathway (with some probability)



Arkin *et al.* (1998), *Genetics*, 149(4): 1633-1648

Comparison with deterministic approach

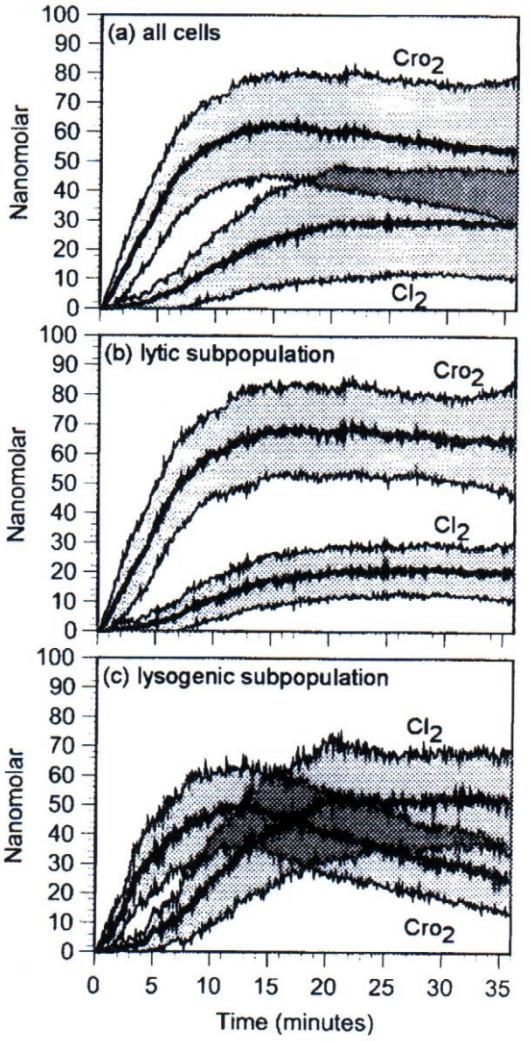
- Deterministic models can be seen as predicting **average behavior** of cell population

  Gillespie. (2000), *J. Chem. Phys.*, 113(1): 297-306

- Analysis of average behavior may obscure that one part of population chooses one pathway rather than another

  Arkin *et al.* (1998), *Genetics*, 149(4): 1633-1648

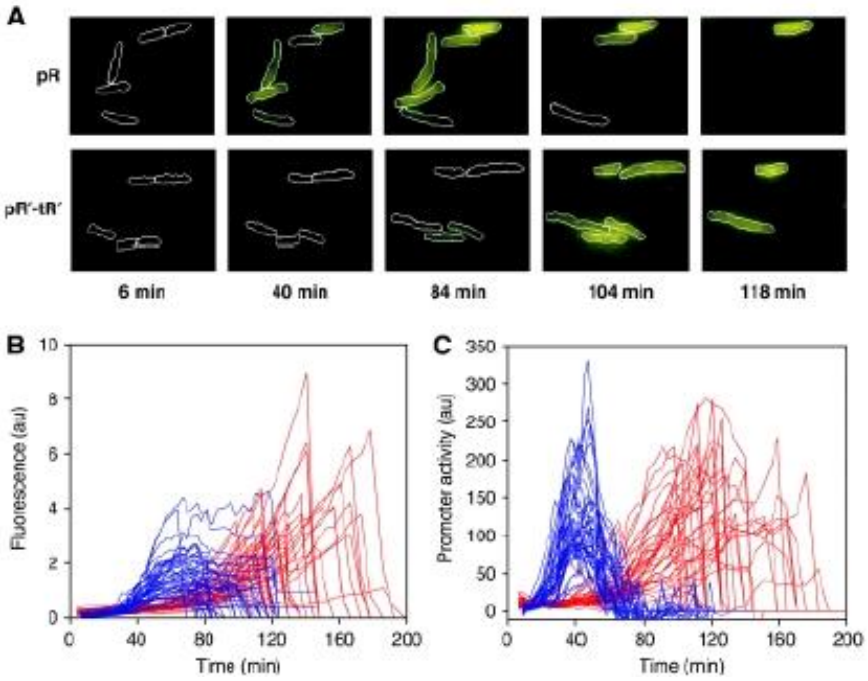
- However, under some conditions deterministic models yield good approximation



Measurements of phage λ infection

- New measurement techniques allow real-time and *in-vivo* monitoring of the execution of lytic and lysogenic pathways in individual cells

  Use of reporter genes in combination with fluorescence microscopy

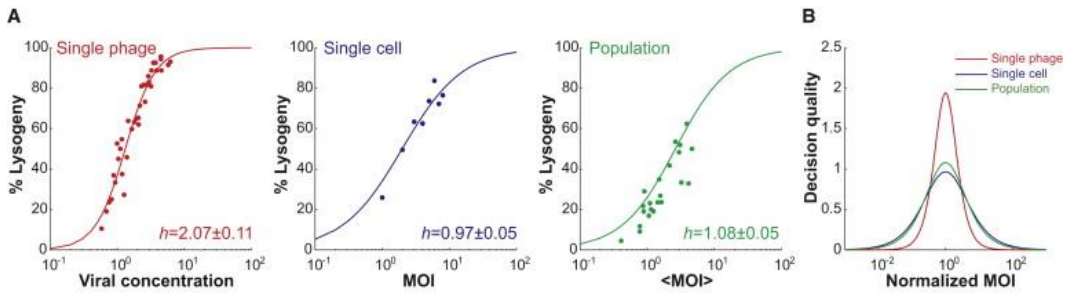


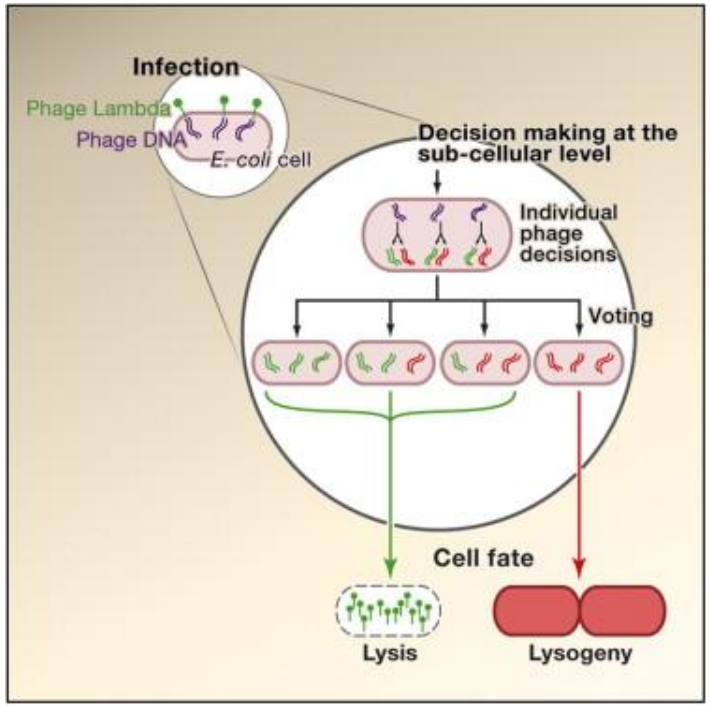
Amir *et al.* (2007), *Mol. Syst. Biol.*, 3:71

Stochasticity and hidden variables

- Is observed population heterogeneity entirely due to stochastic dynamics of biochemical reactions?
- **Hidden variables** that deterministically set outcome of what seems noisy decision process

  Deterministic voting of stochastic decision in single phages





Zeng *et al.* (2010), *Cell*, 141(4):682-91

Conclusions

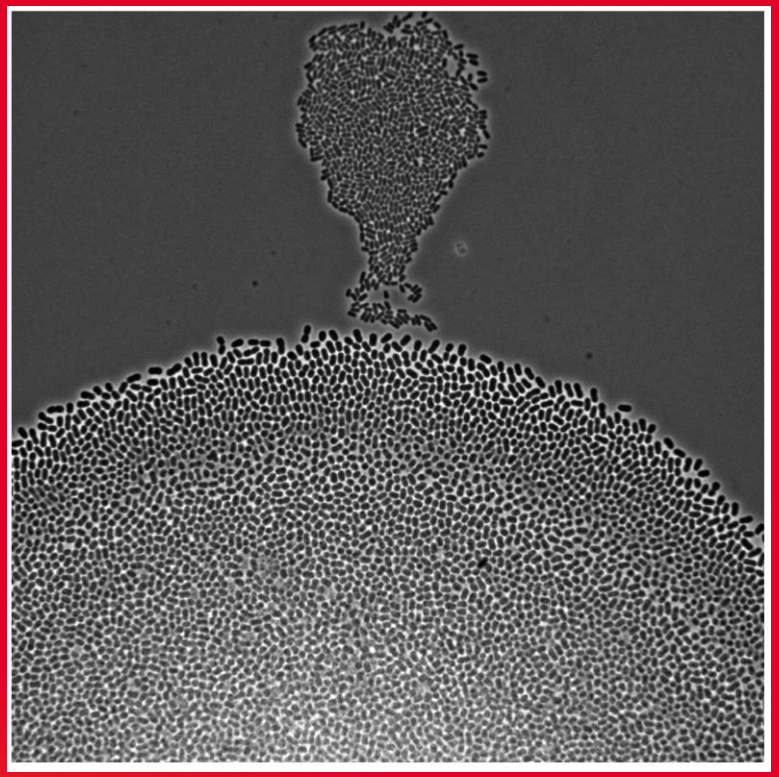
- Stochastic models provide more realistic picture of gene expression
- Difficulty of stochastic models is that required information on regulatory mechanisms on molecular level usually not available

  Reaction schemas and kinetic constants, necessary for generating values of parameters τ and ρ, are not or incompletely known

- Another difficulty is that stochastic simulation is computationally expensive

  Large networks cannot currently be handled, but a host of extensions and approximations have been developed

Thanks!



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