Simulation of Chemical Reactions

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Gillespie's algorithm

Simulation of chemical reactions

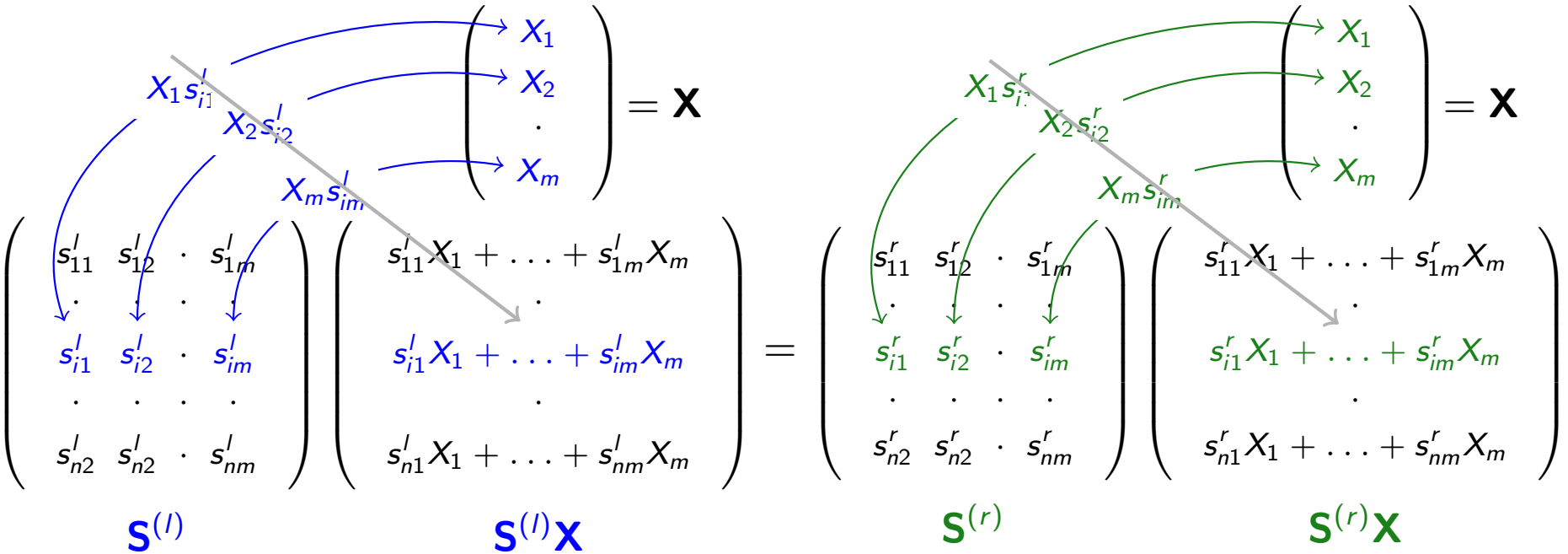
- Chemical system with m reactant types and n possible reactions
- Reactant quantities change over time as reactions occur
- Nr. of type j reactants at time t denoted as $X_j(t)$
- System's state $\Rightarrow$ vector $\mathbf{X}(t) := [X_1(t), X_2(t), \ldots, X_j(t)]^T$
- To specify i-th reaction $\Rightarrow$ reactants, products and rates

$$R_i : s_{i1}^l X_1 + s_{i2}^l X_2 + \ldots + s_{im}^l X_m \overset{h_i(\mathbf{X})}{\rightarrow} s_{i1}^r X_1 + s_{i2}^r X_2 + \ldots + s_{im}^r X_m$$

- (s_{i1}^l molecules of type 1) $+ \ldots +$ (s_{im}^l molecules of type m) react ... ... to yield (s_{i1}^r of type 1) $+ \ldots +$ (s_{im}^r of type m)
- Rate of reaction $h_i(\mathbf{X})$ depends on number of molecules present
- Let $T_i(\mathbf{X})$ denote the time until the i-th reaction when state is $\mathbf{X}$

Stoichiometry matrices

- Can be more conveniently written using matrices
  - $\Rightarrow$ Define vector of rates $\mathbf{h}(\mathbf{X}) = [h_1(\mathbf{X}), h_2(\mathbf{X}), \ldots, h_n(\mathbf{X})]^T$
  - $\Rightarrow$ Define stoichiometry left matrix $\mathbf{S}^{(l)}$ with elements s_{ij}^l
  - $\Rightarrow$ Define stoichiometry right matrix $\mathbf{S}^{(r)}$ with elements s_{ij}^r
- Write system of chemical reactions as $\Rightarrow \mathbf{S}^{(l)}\mathbf{X} \xrightarrow{\mathbf{h}(\mathbf{X})} \mathbf{S}^{(r)}\mathbf{X}$



Example 1: Dimerization kinetics

- Molecule can exist in simple form P and as a dimer D
- Define vector $\mathbf{X} := [P, D]^T$
- Possible reactions are dimerization and dissociation

$$R_1 \text{ (Dimerization): } 2P \overset{h_1(\mathbf{X})}{\rightarrow} D$$

$$R_2 \text{ (Dissociation): } D \overset{h_2(\mathbf{X})}{\rightarrow} 2P$$

- Rates and stoichiometry matrices $\mathbf{S}^{(l)}$ and $\mathbf{S}^{(r)}$ given by

$$\mathbf{S}^{(l)} = \begin{bmatrix} 2 & 0 \\ 0 & 1 \end{bmatrix}, \quad \mathbf{S}^{(r)} = \begin{bmatrix} 0 & 1 \\ 2 & 0 \end{bmatrix}, \quad \mathbf{h}(\mathbf{X}) = \begin{bmatrix} h_1(\mathbf{X}) \\ h_2(\mathbf{X}) \end{bmatrix}$$

- Rewrite equations more compactly as $\Rightarrow \mathbf{S}^{(l)}\mathbf{X} \overset{\mathbf{h}(\mathbf{X})}{\rightarrow} \mathbf{S}^{(r)}\mathbf{X}$

Example 2: Enzymatic reaction

- Substrate S converted to product P. Enzyme E catalyzes conversion
- Converting S into P directly requires significant energy
- Enzyme E reacts with S to form intermediate molecule SE (binding)
- Molecule SE then separates into product P liberating E (conversion)
- This cycle requires less energy than direct conversion
- SE may also separate back into S and E (dissociation)

- Possible reactions are binding, conversion and dissociation, then

$$R_1 \text{ (Binding):} \quad S + E \overset{h_1(\mathbf{X})}{\rightarrow} SE$$

$$R_2 \text{ (Dissociation):} \quad SE \overset{h_2(\mathbf{X})}{\rightarrow} S + E$$

$$R_3 \text{ (Conversion):} \quad SE \overset{h_3(\mathbf{X})}{\rightarrow} E + P$$

Example 2: Enzymatic reaction (continued)

- System state represented by vector $\mathbf{X} := [S, E, SE, P]^T$
- Stoichiometry matrices $\mathbf{S}^{(l)}$ and $\mathbf{S}^{(r)}$ given by

$$\mathbf{S}^{(l)} = \begin{array}{c} \begin{matrix} S & E & SE & P \end{matrix} \\ \begin{bmatrix} 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 \end{bmatrix} \end{array} \begin{matrix} R_1 \\ R_2 \\ R_3 \end{matrix} \qquad \mathbf{S}^{(r)} = \begin{array}{c} \begin{matrix} S & E & SE & P \end{matrix} \\ \begin{bmatrix} 0 & 0 & 1 & 0 \\ 1 & 1 & 0 & 0 \\ 0 & 1 & 0 & 1 \end{bmatrix} \end{array} \begin{matrix} R_1 \\ R_2 \\ R_3 \end{matrix}$$

- Reaction rate vector $\mathbf{h}(\mathbf{X}) = [h_1(\mathbf{X}), h_2(\mathbf{X}), h_3(\mathbf{X})]^T$
- Rewrite equations more compactly as $\Rightarrow \mathbf{S}^{(l)}\mathbf{X} \overset{\mathbf{h}(\mathbf{X})}{\rightarrow} \mathbf{S}^{(r)}\mathbf{X}$

Second order reaction

- Consider second order reaction $R_i : X_1 + X_2 \to \ldots$ (two reactants)
- Let $T_i(X_1, X_2)$ be time until R occurs when there are X_1 type 1 and X_2 type 2 molecules
- Have seen that $T_i(X_1, X_2)$ is exponentially distributed with rate

$$h_i(\mathbf{X}) = h_i(X_1, X_2) = c_i X_1 X_2$$

- Constant c_i measures reactivity of X_1 and X_2
- Argument $\Rightarrow$ $T_i(1,1)$ memoryless (depends on chance encounter)
  $\Rightarrow$ Thus $T_i(1,1)$ is exponential with, say, parameter c_i
  $\Rightarrow$ $T_i(X_1, X_2)$ is the minimum of $X_1 X_2$ exponentials
  $\Rightarrow$ $T_i(X_1, X_2)$ exponential with parameter $c_i X_1 X_2$

Second order involving molecules of same type

- Second order reaction with two molecules of same type

$$R_i : X_1 + X_1 \to \dots$$

- Hazard depends on the number of molecules X_1, i.e. $h_i(\mathbf{X}) = h_i(X_1)$
- Reaction does not occur if there is a single molecule
- If there are 2 molecules $T_i(2)$ is exponential with parameter, say, c_i
- For arbitrary X_1 there are $X_1(X_1 - 1)/2$ possible encounters
- Then, $T_i(X_1)$ is exponential with parameter

$$h_i(\mathbf{X}) = h_i(X_1) = c_i X_1(X_1 - 1)/2$$

- $c_i X_1(X_1 - 1)/2$ substantially different from $c_i X_1^2/2$ for small X_1

Zero-th, first and higher order reactions

- Zero-th order reaction $R_i : \emptyset \to X_1$ (spontaneous generation)
- Assume an exponential model with constant rate $h_i = c_i$
- Used to model exogenous factors (and biblical phenomena)

- First order reaction $R_i : X_1 \to \ldots$ (decay)
- Exponential with rate $h_i(\mathbf{X}) = h_i(X_1) = c_i X_1$

- Higher order reactions involving more than two reactants
- E.g., third order reaction $R_i : X_1 + X_2 + X_3 \to X_4$
- Time until next R_i reaction exponential. Hazard: $h_i(\mathbf{X}) = c_i X_1 X_2 X_3$
- Reactions of order more than 2 are rare
- Most likely, R_i is encapsulating two second order reactions

$$X_1 + X_2 \to X_5, \qquad X_5 + X_3 \to X_4$$

The hazard function

- All reaction times are exponential RVs $\Rightarrow$ CTMC with state $\mathbf{X}$
- Hazards $h_i(\mathbf{X})$ determine transition rates of CTMC
- Hazards for zero-th, first and second order reactions (for reference)

Order	Reaction		Rate
zero-th	$\emptyset$	$\xrightarrow{c} \cdots$	c
first	X_1	$\xrightarrow{c} \cdots$	cX_1
second	$X_1 + X_2$	$\xrightarrow{c} \cdots$	cX_1X_2
second	$2X_1$	$\xrightarrow{c} \cdots$	$cX_1(X_1 - 1)/2$

- Probability of reaction R_i happening in infinitesimal time ϵ is

$$\mathrm{P}\left(T_i(\mathbf{X}) < \epsilon\right) = h_i(\mathbf{X})\epsilon + o(\epsilon)$$

- That's why the name hazard

State transition for given reaction

- State is $\mathbf{X}(t) = \mathbf{X}$. Reaction R_i occurs. Next state $\mathbf{X}(t + dt) = \mathbf{Y}$?
- Number of reactants per type =
  $= i$-th row of left stoichiometry matrix $\mathbf{s}_i^{(l)} = [s_{i1}^l, s_{i2}^l, \ldots, s_{im}^l]^T$

$$s_{i1}^l X_1 + s_{i2}^l X_2 + \ldots + s_{im}^l X_m \overset{h_i(\mathbf{X})}{\rightarrow} \ldots$$

- Number of products per type =
  $= i$-th row of right stoichiometry matrix $\mathbf{s}_i^{(r)} = [s_{i1}^r, s_{i2}^r, \ldots, s_{im}^r]^T$

$$\ldots \overset{h_i(\mathbf{X})}{\rightarrow} s_{i1}^r X_1 + s_{i2}^r X_2 + \ldots + s_{im}^r X_m$$

- X decreases by nr. of reactants and increases by nr. of products
- Next sate is $\Rightarrow \mathbf{Y} = \mathbf{X} - \mathbf{s}_i^{(l)} + \mathbf{s}_i^{(r)}$ (upon reaction R_i)

Transition rates and probabilities

- $q(\mathbf{X}, \mathbf{Y})$ = transition rate from state $\mathbf{X}$ to state $\mathbf{Y}$. Given by

$$q\left(\mathbf{X}, \mathbf{X} - \mathbf{s}_i^{(l)} + \mathbf{s}_i^{(r)}\right) = h_i(\mathbf{X}), \quad i = 1, \ldots, n$$

- Transition from state $\mathbf{X}$ to $\mathbf{X} - \mathbf{s}_i^{(l)} + \mathbf{s}_i^{(r)}$ when reaction R_i occurs
- $\nu(\mathbf{X})$ = Transition rate out of $\mathbf{X}$ into any state (any reaction occurs)

$$\nu(\mathbf{X}) = \sum_{i=1}^{n} q\left(\mathbf{X}, \mathbf{X} - \mathbf{s}_i^{(l)} + \mathbf{s}_i^{(r)}\right) = \sum_{i=1}^{n} h_i(\mathbf{X})$$

- $P(\mathbf{X}, \mathbf{Y})$ = Prob. of going into $\mathbf{Y}$ given transition out of $\mathbf{X}$ occurs

$$P\left(\mathbf{X}, \mathbf{X} - \mathbf{s}_i^{(l)} + \mathbf{s}_i^{(r)}\right) = \frac{q\left(\mathbf{X}, \mathbf{X} - \mathbf{s}_i^{(l)} + \mathbf{s}_i^{(r)}\right)}{\nu(\mathbf{X})} = \frac{h_i(\mathbf{X})}{\nu(\mathbf{X})}$$

- Probability that i-th reaction occurs given that a reaction occurred

Gillespie's algorithm

Gillespie's algorithm = Simulation of CTMC

Input: Stoichiometry matrices $\mathbf{S}^{(l)}$ and $\mathbf{S}^{(r)}$. Initial state $\mathbf{X}(0)$

Output: Molecule numbers as a function of time $\mathbf{X}(t)$

(1) Initialize time and CTMC's state $t = 0,\ \mathbf{X} = \mathbf{X}(0)$

(2) Calculate all hazards $\Rightarrow h_i(\mathbf{X})$

(3) Calculate transition rate $\Rightarrow \nu(\mathbf{X}) = \sum_{i=1}^{n} h_i(\mathbf{X})$

(4) Draw random time of next reaction $\Delta t \sim \text{Exp}\big(\nu(\mathbf{X})\big)$

(5) Advance time to $t = t + \Delta t$

(6) Draw reaction at time $t + \Delta t$ $\Rightarrow$ R_i drawn with prob. $h_i(\mathbf{X})/\nu(\mathbf{X})$

(7) Update state vector to account for this reaction $\Rightarrow \mathbf{X} - \mathbf{s}_i^{(l)} + \mathbf{s}_i^{(r)}$

(8) Repeat from (2)

Dimerization Kinetics

Gillespie's algorithm

Dimerization Kinetics

Enzymatic Reactions

Lactose digestion (lac operon)

Dimerization

- Dimerization occurs when two like molecules join together
- Many proteins (P) will form dimers (D)
- Dimerization may be rare in relative terms, but significant in absolute terms at high concentration. For this reason plays important role in auto-regulation of protein production
- Possible reactions are dimerization and dissociation

$$R_1 \text{ (Dimerization): } 2P \xrightarrow{c_1} D$$

$$R_2 \text{ (Dissociation): } D \xrightarrow{c_2} 2P$$

- Dimerization rare and dimers unstable $\Rightarrow c_2 \gg c_1$
- Stoichiometry matrices $\mathbf{S}^{(l)}$ and $\mathbf{S}^{(r)}$ given by

$$\mathbf{S}^{(l)} = \begin{bmatrix} 2 & 0 \\ 0 & 1 \end{bmatrix}, \quad \mathbf{S}^{(r)} = \begin{bmatrix} 0 & 1 \\ 2 & 0 \end{bmatrix},$$

- Rate of reaction 1 is $h_1(\mathbf{X}) = c_1 P(P-1)/2$. Reaction 2 is $h_2(\mathbf{X}) = c_2 D$

Gillespie's algorithm for dimerization kinetics

(1) Initialize time and CTMC's state $t = 0$, $P = P(0)$, $D = D(0)$

(2) Calculate hazards $\Rightarrow h_1(\mathbf{X}) = c_1 P(P-1)/2$,
$\Rightarrow h_2(\mathbf{X}) = c_2 D$

(3) Calculate transition rate $\Rightarrow \nu(\mathbf{X}) = c_1 P(P-1)/2 + c_2 D$

(4) Draw random time of next reaction

$$\Delta t \sim \exp\left(\nu(\mathbf{X})\right) = \exp\left(c_1 P(P-1)/2 + c_2 D\right)$$

(5) Advance time to $t = t + \Delta t$

(6) Draw reaction at time $t + \Delta t$

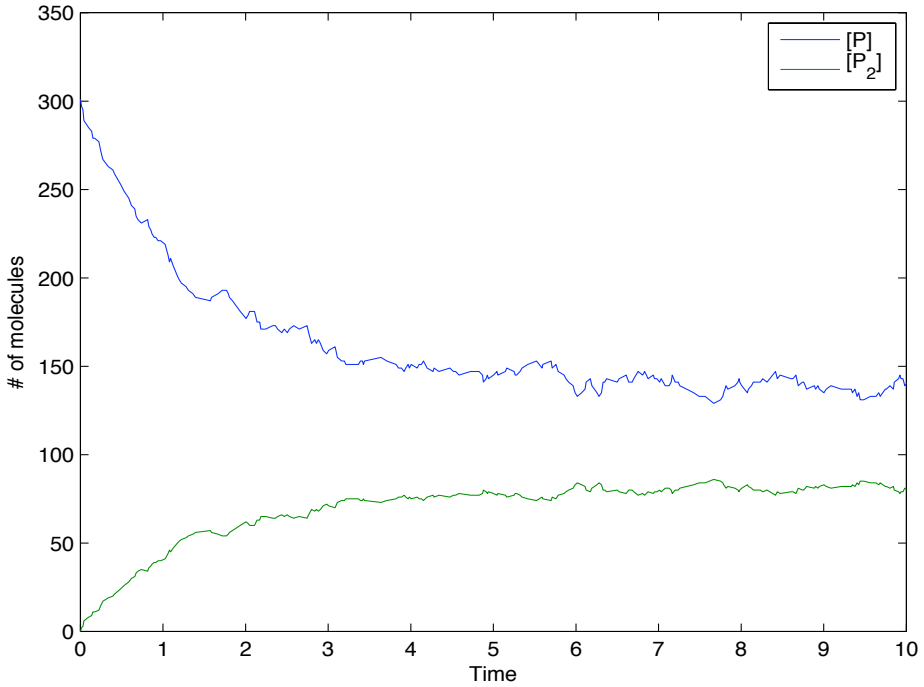
$$\begin{aligned} \mathrm{P}\left(\text{Dimerization:}\right) &= c_1 P(P-1)/2/\nu(\mathbf{X}) \\ \mathrm{P}\left(\text{Dissociation:}\right) &= c_2 D/\nu(\mathbf{X}) \end{aligned}$$

(7) Update state vector $\Rightarrow$ Dimerization: $P = P - 2$, $D = D + 1$
$\Rightarrow$ Dissociation: $P = P + 2$, $D = D - 1$

(8) Repeat from (2)

Stochastic simulation of dimerization kinetics

- Run of Gillespie's algorithm for dimerization kinetics
- Initial condition $P(0) = 301$, $D(0) = 0$ (protein only)



- Dimerization hazard

$$c_1 = 1.66 \times 10^{-3} \frac{\text{reactions}}{\text{sec./molecule}^2}$$

- Dissociation hazards

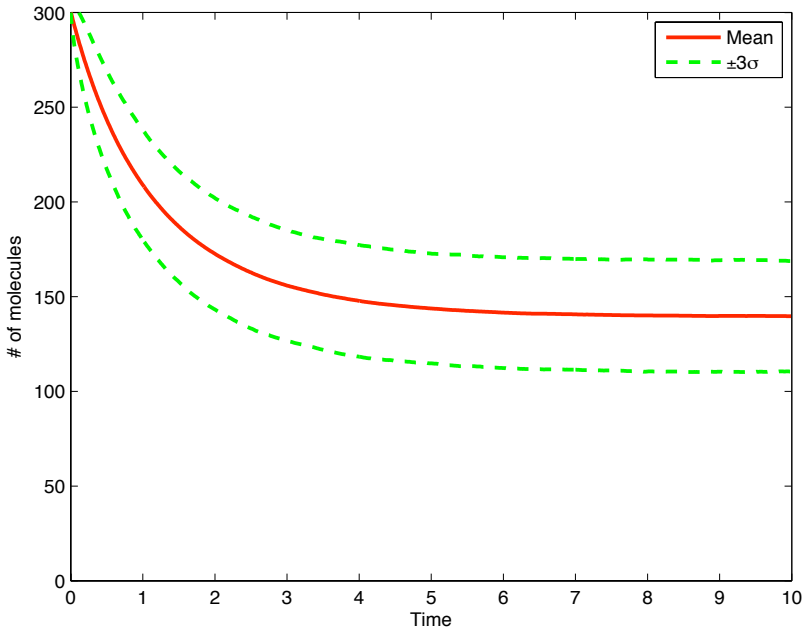
$$c_2 = 0.2 \times 10^{-3} \frac{\text{reactions}}{\text{sec./molecule}}$$

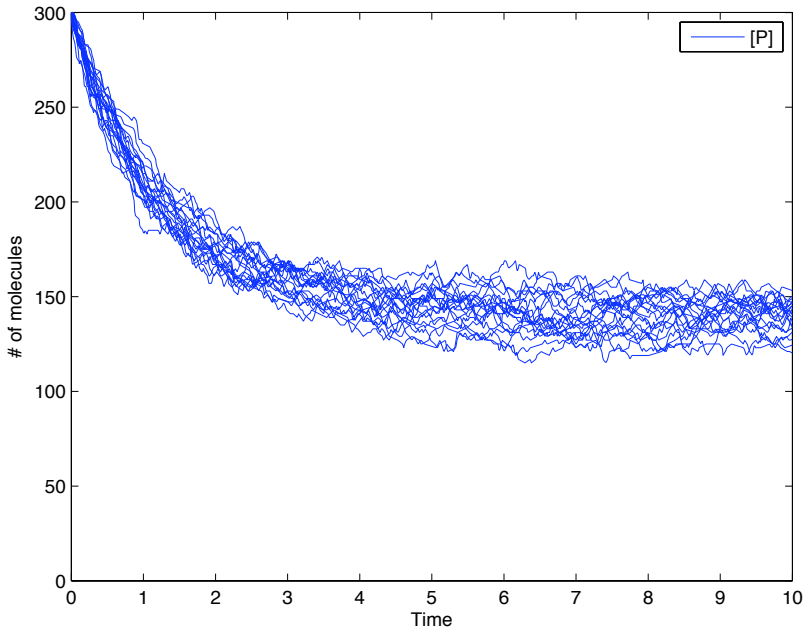
- $\mathbf{c} = [c_1, c_2]^T = [1.66 \times 10^{-3}, 0.2]^T$

- P and D "stabilize" at point where dimerization and dissociation become equally likely

Information that can be obtained from simulations

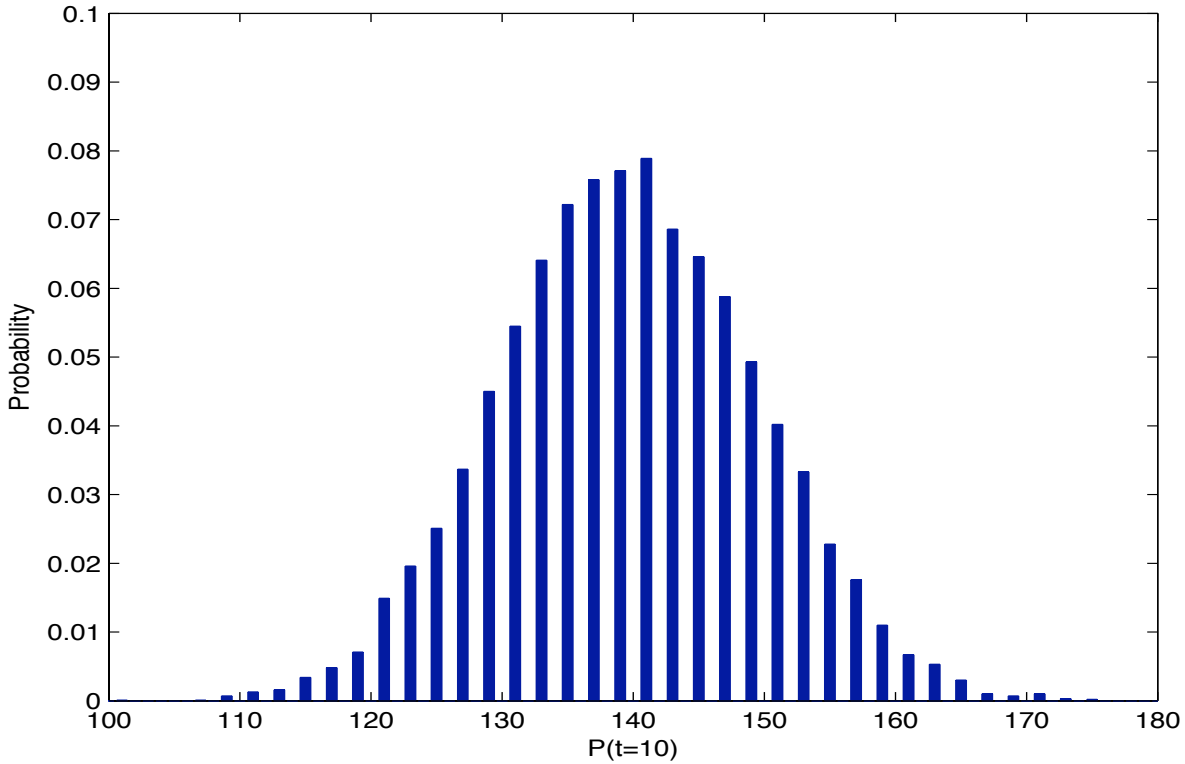
- E.g., consider nr. of protein molecules P ($P(t) + 2D(t)$ is constant)
- Mean and standard deviation of P versus time?
- Right graph $\Rightarrow$ mean and ± 3(standard deviations) over 10^4 trials
- Left graph shows 20 trials
  - Vary around mean path but stay within ± 3-standard deviations





Steady-state probability distribution

- Time $t = 10$ seconds $\Rightarrow$ approximate PMF over 10^4 trials
- Can use ergodicity instead



- Bell-shaped. Only odd values of P are possible
- Runs are all odd or all even depending on initial condition

Enzymatic reactions

Gillespie's algorithm

Dimerization Kinetics

Enzymatic Reactions

Lactose digestion (lac operon)

Enzymes

- Substrate S converted into product P by action of enzyme E
- Intermediate product SE generated by combination of E and S
- SE later separates into product P liberating the enzyme E
- SE may also dissociate into S and E
- Enzymes can act as catalysts for reactions that would otherwise rarely or never take place

- Possible reactions are binding, dissociation and conversion

$$
\begin{aligned}
R_1 \text{ (Binding):} \quad & S + E \xrightarrow{c_1} SE \\
R_2 \text{ (Dissociation):} \quad & SE \xrightarrow{c_2} S + E \\
R_3 \text{ (Conversion):} \quad & SE \xrightarrow{c_3} P + E
\end{aligned}
$$

- Dissociation typically not significant because $c_2 \ll c_3$

Enzymatic reactions (continued)

- Stoichiometry matrices $\mathbf{S}^{(l)}$ and $\mathbf{S}^{(r)}$ given by

$$\mathbf{S}^{(l)} = \begin{array}{c} \begin{matrix} S & E & SE & P \end{matrix} \\ \begin{bmatrix} 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 \end{bmatrix} \end{array} \begin{matrix} R_1 \\ R_2 \\ R_3 \end{matrix} \qquad \mathbf{S}^{(r)} = \begin{array}{c} \begin{matrix} S & E & SE & P \end{matrix} \\ \begin{bmatrix} 0 & 0 & 1 & 0 \\ 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 \end{bmatrix} \end{array} \begin{matrix} R_1 \\ R_2 \\ R_3 \end{matrix}$$

- Reaction rates are
  - $\Rightarrow$ Reaction R_1 (Binding): $h_1(\mathbf{X}) = c_1 S \times E$,
  - $\Rightarrow$ Reaction R_2 (Dissociation): $h_2(\mathbf{X}) = c_2 SE$
  - $\Rightarrow$ Reaction R_3 (Conversion): $h_3(\mathbf{X}) = c_3 SE$

Gillespie's algorithm for enzymatic reactions

(1) Initialization: $t = 0$, $S = S(0)$, $E = E(0)$, $SE = SE(0)$, $P = P(0)$

(2) Calculate hazards $\Rightarrow h_1(\mathbf{X}) = c_1 S \times E$,
$\Rightarrow h_2(\mathbf{X}) = c_2 SE$
$\Rightarrow h_3(\mathbf{X}) = c_3 SE$

(3) Calculate transition rate $\Rightarrow \nu(\mathbf{X}) = c_1 S \times E + c_2 SE + c_3 SE$

(4) Draw random time of next reaction

$$\Delta t \sim \exp\left(\nu(\mathbf{X})\right) = \exp\left(c_1 S \times E + c_2 SE + c_3 SE\right)$$

(5) Advance time to $t = t + \Delta t$

(6) Draw reaction at time $t + \Delta t$

$$\begin{aligned} \mathrm{P}\,(\text{Binding:}) &= c_1 S \times E / \nu(\mathbf{X}) \\ \mathrm{P}\,(\text{Dissociation:}) &= c_2 SE / \nu(\mathbf{X}) \\ \mathrm{P}\,(\text{Conversion:}) &= c_3 SE / \nu(\mathbf{X}) \end{aligned}$$

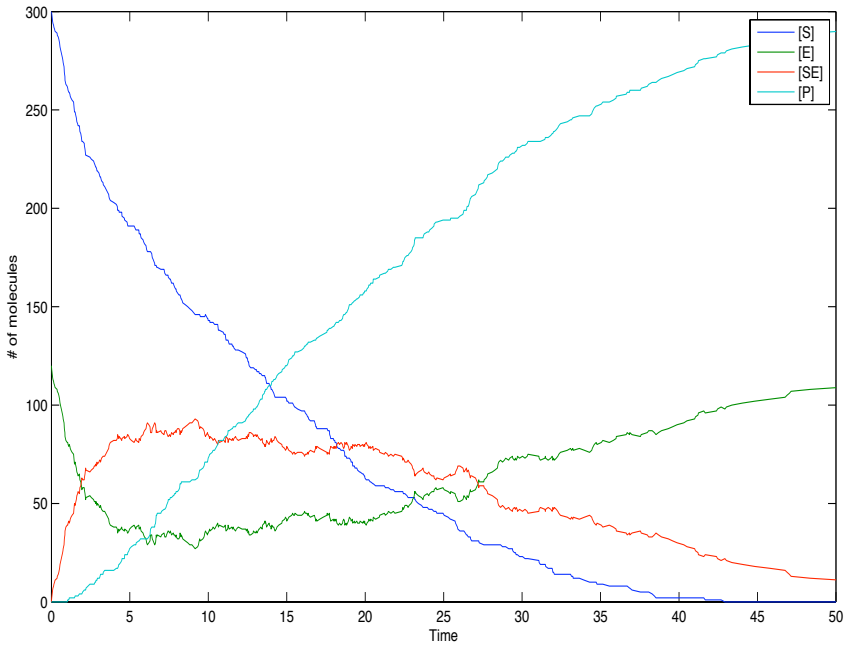
(7) Update state vector $\Rightarrow$ Binding: $S = S - 1$, $E = E - 1$, $SE = SE + 1$
$\Rightarrow$ Dissociation: $S = S + 1$, $E = E + 1$, $SE = SE - 1$
$\Rightarrow$ Conversion: $P = P + 1$, $E = E + 1$, $SE = SE - 1$

(8) Repeat from (2)

Stochastic simulation of enzymatic reactions

- Run of Gillespie's algorithm for enzymatic reactions
- Initialize with only substrate and enzyme present

$$S(0) = 301,\ E(0) = 120,\ SE(0) = 0,\ P(0) = 0$$



- Binding hazard

$$c_1 = 1.66 \times 10^{-3} \frac{\text{reactions}}{\text{sec./molecule}^2}$$

- Dissociation hazard

$$c_2 = 10^{-4} \frac{\text{reactions}}{\text{sec./molecule}}$$

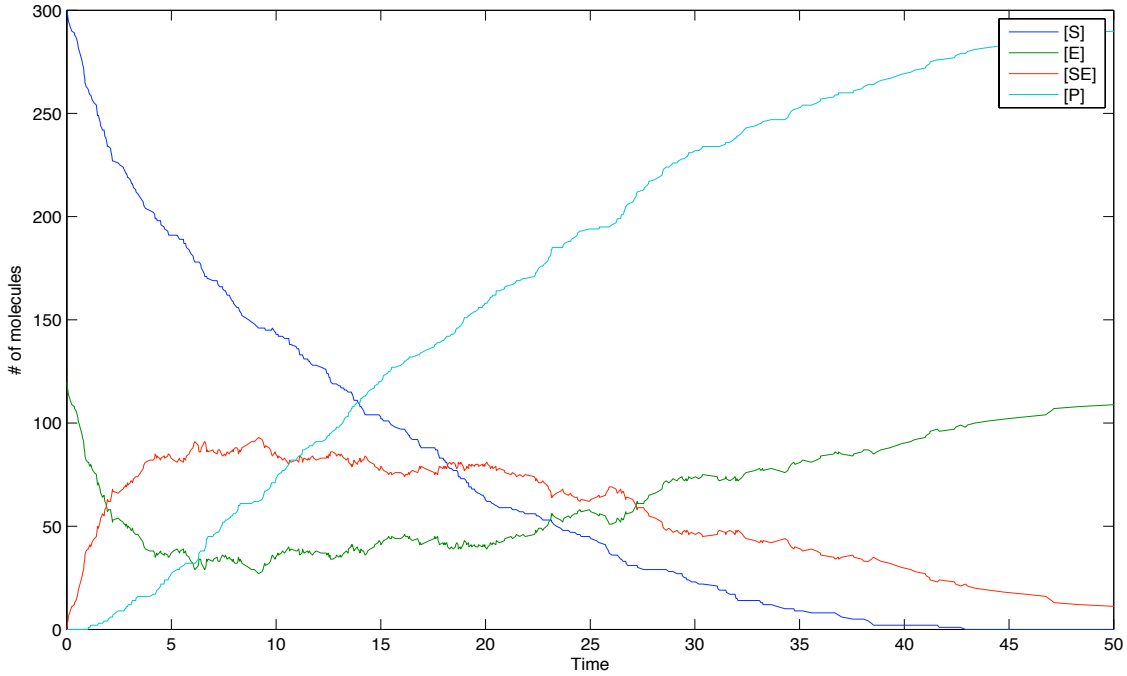
- Conversion hazard

$$c_3 = 0.1 \frac{\text{reactions}}{\text{sec./molecule}}$$

- $\mathbf{c} = [c_1, c_2, c_3]^T = [1.66 \times 10^{-3}, 10^{-4}, 0.1]^T$

Stochastic simulation (continued)

- At the beginning substrate and enzyme numbers decline as they bind to each other to form intermediate product SE
- Intermediate product separates into final product P liberating enzyme E
- By $t = 50$ seconds substrate is completely converted into product and enzymes are free. There is no intermediate product either



Lactose digestion (lac operon)

Gillespie's algorithm

Dimerization Kinetics

Enzymatic Reactions

Lactose digestion (lac operon)

Auto-regulation of protein production

- Simplified model of protein production in prokaryotes
- "Instructions" for creating proteins "encoded" in genes
- To produce proteins, genes are first transcribed into mRNA
- This mRNA is passed on to a ribosome to "assemble" the protein

- Protein production not immutable. How does it changes over time?
- Auto regulatory gene networks
  - $\Rightarrow$ Production triggered by external stimuli
  - $\Rightarrow$ Halted by negative feedback loops through protein byproducts

- E.g. Production of β-galactosidase to digest glucose
  - $\Rightarrow$ Lac-operon (lac for lactose, operon=set of interacting genes)

Glucose, Lactose and β-galactosidase

- Glucose (G) and lactose (L) are variations of sugars
- Cells use glucose for energy but can reduce lactose to glucose
- Lactose reduced to glucose by enzyme β-galactosidase (βG)

$$\text{Lactose digestion:} \quad L + \beta G \xrightarrow{c_1} G + \beta G$$

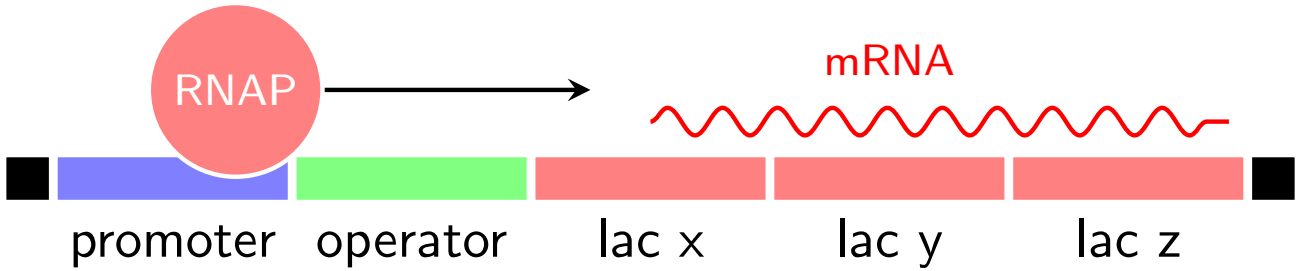
$$\text{Glucose consumption:} \quad G \xrightarrow{c_2} \emptyset$$

- Did not model enzymatic reaction (compare with earlier example)
- Rate of lactose digestion $c_1 L \times (\beta G)$. Glucose consumption $c_2 G$
- Producing β-galactosidase is not always necessary
- Production necessary only when lactose is present and glucose is not

Lac-operon, normal state

- Lac-operon consists of three adjacent genes
- Promoter, operator and β-galactosidase code (three types in fact)
- Lac-operon has three possible states, regular, activated and repressed

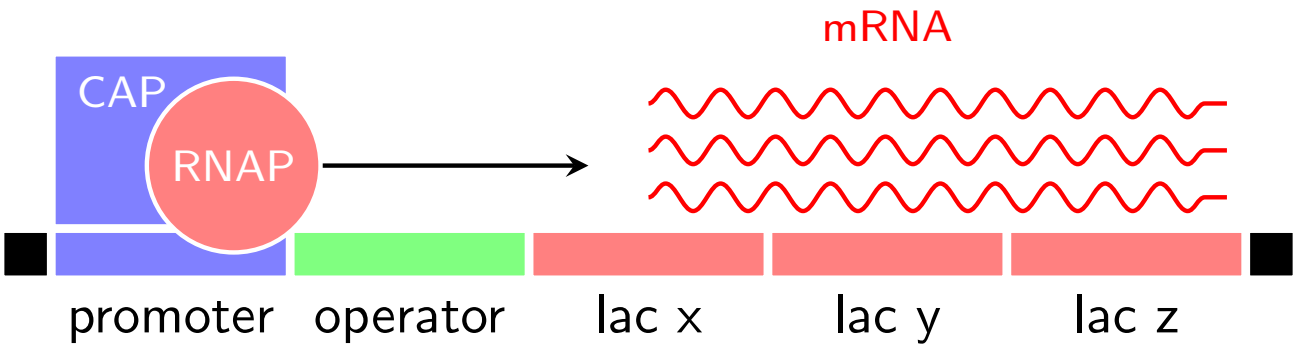
- In normal state (Op) transcription proceeds at a small rate c_3
- The promoter is a binding place for RNA polymerase (RNAP)
- RNAP binds to promoter to initiate gene transcription into mRNA



- Model reaction as $\Rightarrow$ Regular transcription: $Op \xrightarrow{c_3} Op + mRNA$

Lac-operon in activated state

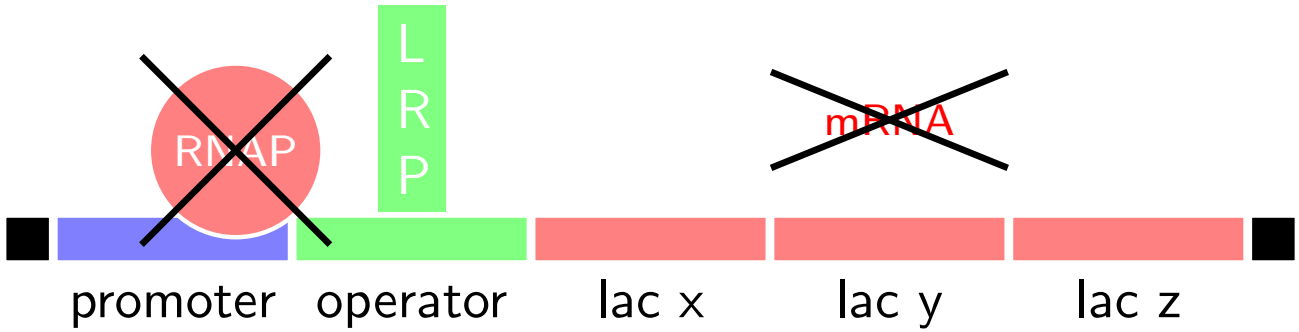
- Operon activated (AOp) by catabolite activator protein (CAP)
- CAP binds upstream of the promoter altering DNA's geometry
- Thereby facilitating (promoting) binding of RNAP to promoter
- Hence yielding a faster rate of transcription $c_4 \gg c_3$



- Model reaction as $\Rightarrow$ Activated transcription: $AOp \xrightarrow{c_4} AOp + mRNA$

Lac-operon in repressed state

- Operon repressed (ROp) by lactose repressor protein protein (LRP)
- LRP encoded by gene adjacent to lac operon, is always expressed and has great affinity with the operator
- If LRP binds to operator it interferes with RNAP–promoter binding
- Without RNAP, there is no (or minimal) transcription
- Hence yielding a very slow rate of transcription $c_5 \ll c_3 \ll c_4$

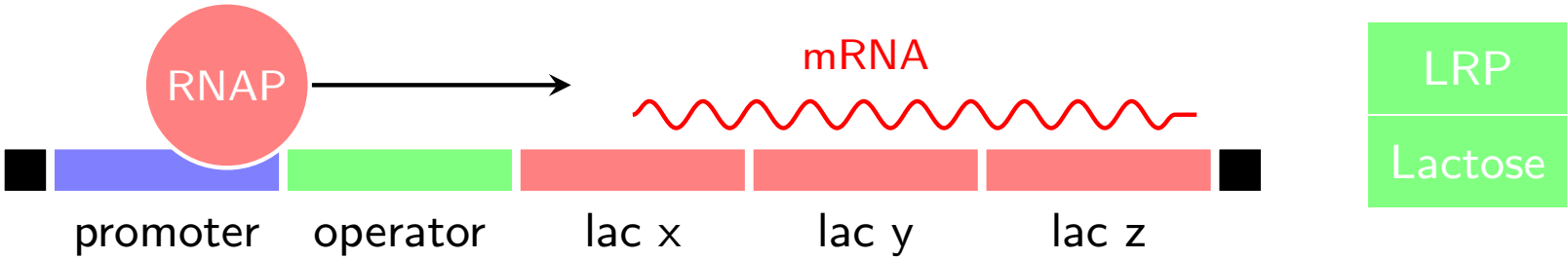


- Model reaction as $\Rightarrow$ Repressed transcription: $ROp \xrightarrow{c_5} ROp + mRNA$

Repression control

- If there is no lactose (L) present lac operon is in repressed state
- When lactose is present it combines with LRP
- Thereby preventing repression of lac operon. Lac operon in regular state

  $\Rightarrow$ Small (but not minimal) rate of β-galactosidase production



- We model this with the following reactions

$$\begin{aligned}
&\text{Operon repression:} && LRP + Op \xrightarrow{c_6} ROp \\
&\text{Operon liberation:} && ROp \xrightarrow{c_7} LRP + Op \\
&\text{Repressor neutralization:} && LRP + L \xrightarrow{c_8} LRPL \\
&\text{Repressor dissociation:} && LRPL \xrightarrow{c_9} LRP + L
\end{aligned}$$

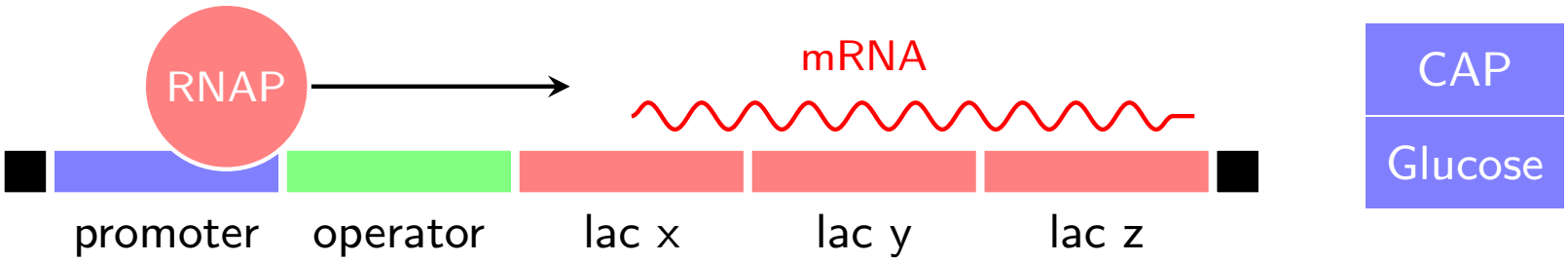
Activation control

- Prevalence of CAP inversely proportional to glucose levels
- This involves a complex set of reactions in itself
- For a preliminary model the following reactions suffice

$$\begin{aligned}
&\text{Operon activation:} && CAP + Op \xrightarrow{c_{10}} AOp \\
&\text{Operon deactivation:} && AOp \xrightarrow{c_{11}} CAP + Op \\
&\text{CAP neutralization:} && CAP + G \xrightarrow{c_{12}} CAPG \\
&\text{CAP dissociation:} && CAPG \xrightarrow{c_{13}} CAP + G
\end{aligned}$$

- If glucose is present, CAP is bound to glucose
- Thereby preventing activation of lac operon

  $\Rightarrow$ Small rate of β-galactosidase production



Glucose, lactose and lac-operon states

- High lactose and high glucose (glucose preferred)
  - CAP bound to glucose and LRP bound to lactose
  - Operon in regular state, low production of β-galactosidase
- High lactose and low glucose (lactose only option)
  - CAP bound upstream of promoter and LRP bound to lactose
  - Operon in activated state, high production of β-galactosidase
- High glucose and low lactose (glucose dominant and preferred)
  - CAP bound to glucose and LRP bound to operator
  - Operon in repressed state, minimal production of β-galactosidase
- Low glucose and low lactose (no energy source available)
  - CAP bound upstream of promoter and LRP bound to operator
  - Repression dominates, minimal production of β-galactosidase
- β-galactosidase produced in significant quantities only with high lactose and low glucose concentrations

β-galactosidase assembly and decays

- To complete model we add reactions to account for
  - $\Rightarrow$ Assembly of β-galactosidase (βG) enzyme
  - $\Rightarrow$ *mRNA* and βG decay

Protein synthesis:	$mRNA \xrightarrow{c_{14}} mRNA + \beta G$
mRNA decay:	$mRNA \xrightarrow{c_{15}} \emptyset$
βgalactosidase decay:	$\beta G \xrightarrow{c_{16}} \emptyset$

Reactions modeling digestion of lactose

- Model of auto-regulatory gene network for digestion of lactose
- Rates in reactions/minute/molecule or reactions/minute/molecule^2

Lactose digestion:	$L + \beta G$	$\xrightarrow{c_1} G + \beta G$	$c_1 = 1$
Glucose consumption:	G	$\xrightarrow{c_2} \emptyset$	$c_2 = 0.1$
Regular transcription:	Op	$\xrightarrow{c_3} Op + mRNA$	$c_3 = 0.01$
Activated transcription:	AOp	$\xrightarrow{c_4} AOp + mRNA$	$c_4 = 0.1$
Repressed transcription:	ROp	$\xrightarrow{c_5} ROp + mRNA$	$c_5 = 0.001$
Operon repression:	$LRP + Op$	$\xrightarrow{c_6} ROp$	$c_6 = 1$
Operon liberation:	ROp	$\xrightarrow{c_7} LRP + Op$	$c_7 = 1$

- Compare rates c_3-c_5 for lac operon in different states

Reactions modeling digestion of lactose (continued)

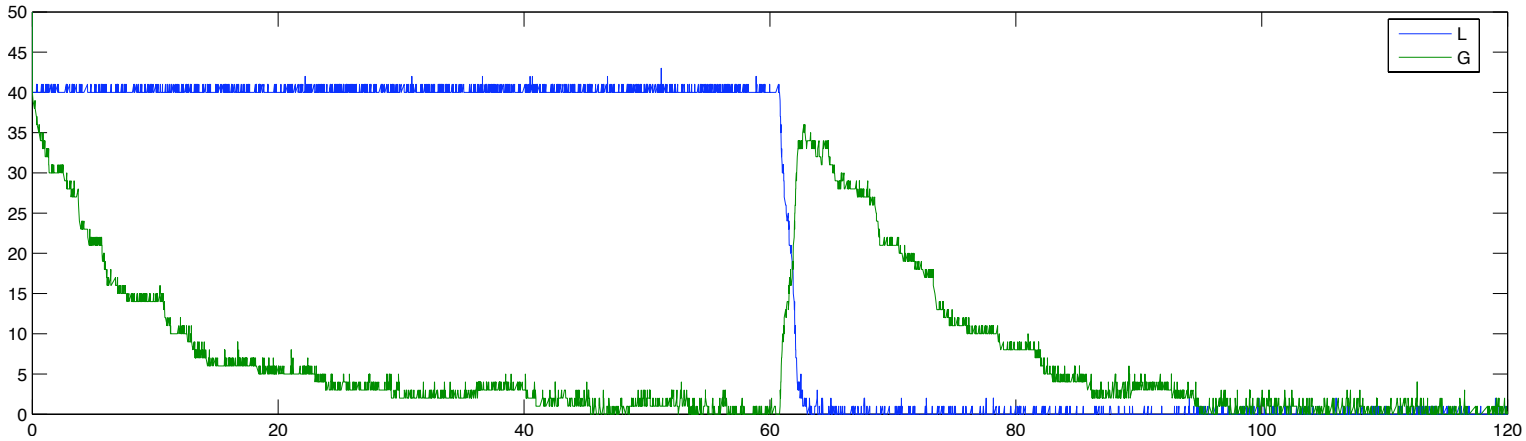
- Model of auto-regulatory gene network for digestion of lactose
- Rates in reactions/minute/molecule or reactions/minute/molecule^2

Repressor neutralization:	$LRP + L$	$\xrightarrow{c_8} LRPL$	$c_8 = 10$
Repressor dissociation:	$LRPL$	$\xrightarrow{c_9} LRP + L$	$c_9 = 1$
Operon activation:	$CAP + Op$	$\xrightarrow{c_{10}} AOp$	$c_{10} = 1$
Operon deactivation:	AOp	$\xrightarrow{c_{11}} CAP + Op$	$c_{11} = 1$
CAP neutralization:	$CAP + G$	$\xrightarrow{c_{12}} CAPG$	$c_{12} = 10$
CAP dissociation:	$CAPG$	$\xrightarrow{c_{13}} CAP + G$	$c_{13} = 1$
Protein synthesis:	$mRNA$	$\xrightarrow{c_{14}} mRNA + \beta G$	$c_{14} = 1$
mRNA decay:	$mRNA$	$\xrightarrow{c_{15}} \emptyset$	$c_{15} = 1$
βgalactosidase decay:	βG	$\xrightarrow{c_{16}} \emptyset$	$c_{16} = 0.1$

- Notice that LRP and CAP neutralization are fast (rates c_8 and c_{12})

Stochastic simulation: diauxie pattern

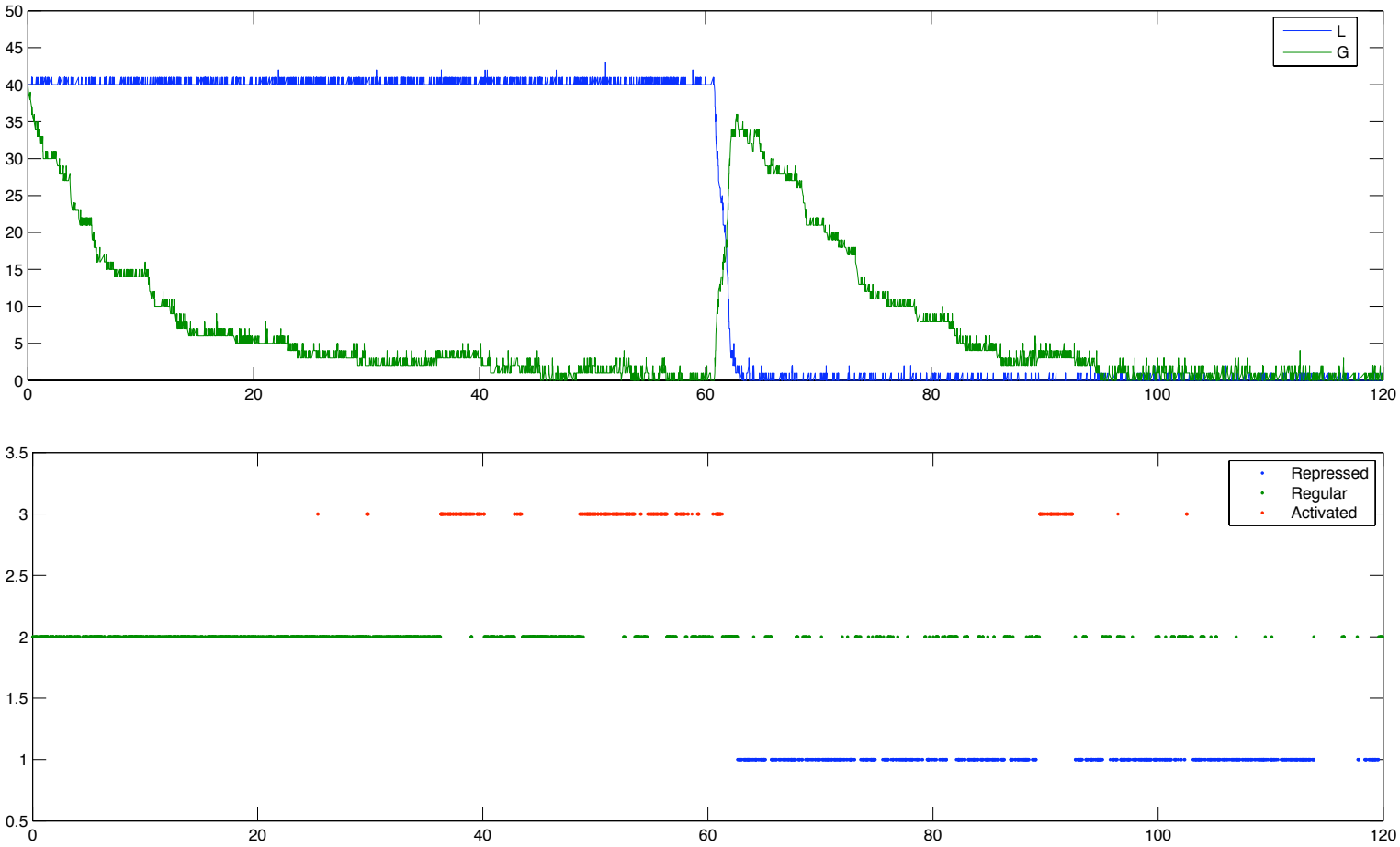
- Initial state $\Rightarrow L = 50,\ G = 50,\ CAP = 10,\ LRP = 10$
- Only 1 operon in regular state



- Sugars (glucose and lactose) consumed sequentially
  - $\Rightarrow$ Glucose is consumed first
  - $\Rightarrow$ After glucose is depleted, lactose converted to glucose
  - $\Rightarrow$ After conversion, newly generated glucose is also consumed
- Yields two growth spurts = diauxie pattern

Operon state and diauxie pattern

- Conversion occurs with operon in activated state



mRNA transcription & β-Galactosidase synthesis

- Operon activation $\Rightarrow$ mRNA transcription $\Rightarrow$ β-Galactosidase synthesis $\Rightarrow$ lactose digestion