Criticality and Adaptivity in Enzymatic Networks

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Enzymes

• Large biological molecules that act as catalysts for complex biochemical reactions in living organisms

\[ S + E \xrightleftharpoons[\eta^-]{\eta^+} [SE] \xrightarrow{\mu} E + P \]

• Deterministic model: Michaelis-Menten equation

\[ \frac{d[P]}{dt} = \frac{\mu[E]_0[S]}{K + [S]} \]

\[ K = \frac{\eta^-}{\eta^+} \]

• Here: stochastic model, limited #enzymes, shared
Bottlenecks in Enzymatic Processing

**Competitive enzymatic degradation in *E. Coli***:

- Mistranslated proteins
- Stress response
- RNA polymerase

**Oxidative stress response in *S. pombe***:

- $\text{H}_2\text{O}_2$
- Stress kinases
- elf2α kinases
- Translation ↓
- Growth ↓
- mRNA ↓
- Potentiation
- Ribosome capacity
- Recovery
- Protein ↑

**Translational crosstalk**:

- galactose
- Clp3p
- mRNA for growth
- mRNA for reproduction
- Protein synthesis
- ATP and building blocks
- cell components and division machinery
- Glucose
- cell division

**Synthetic shared degradation model**:

- TetR
- Dox.
- P_{lacI} YFP-LAA
- AraC
- P_{lacI} CFP-LAA
- ClpXP
Connection to Queueing

- Queueing theory traditionally has used stochastic models to understand congestion effects in man-made systems in engineering and business where the processing resources are limited.
- Queueing theory useful for formulating, analysing and interpreting models.
- Two interesting regimes.
Two Regimes in Queueing

Underloaded

Service rate > arrival rate
Queues are short
Little competition

Overloaded

Service rate < arrival rate
Queues are long
Strong competition

No queue for iPad mini in London, Nov 2, 2012
Photo by Rik Henderson

Photo by Ilze Ziedins
Two Regimes in Queueing

Underloaded

Service rate > arrival rate
Queues are short

Balance: service rate = arrival rate

Overloaded

Service rate < arrival rate
Queues are long

No queue for iPad mini in London, Nov 2, 2012
Photo by Rik Henderson

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Outline

- Competition for common downstream (degradation) enzyme
- Adaptive enzymatic processing
- Enzymatic networks with shared resources
Competition for Enzymatic Processing

Theory

Experiment
Competition for Degradation

- Two uncoupled proteins $X_1$ and $X_2$ are processed downstream by a common enzyme $E$. 

$\lambda_1$ production/arrival  
$\lambda_2$ dilution/reneging  
$\gamma$  

$(L \text{ copies of enzyme } E)$  
$\mu$ degradation/processing
Stochastic Model

Biochemical reaction network: protein species $X_1, X_2$

\begin{align*}
\emptyset & \xrightarrow{\lambda_i} X_i \quad \text{(production)} \\
X_i + E & \xrightarrow{\eta} X_iE \quad \text{(binding of enzyme)} \\
X_iE & \xrightarrow{\mu} E \quad \text{(degradation)} \\
X_iE & \xrightarrow{\gamma} E, \quad X_i \xrightarrow{\gamma} \emptyset \quad \text{(dilution)}
\end{align*}

Assume: exponential reaction times and binding is instantaneous

Key stochastic processes ($i=1,2$):

$Q_i(t) = \text{total number of molecules of species } i \text{ in the system at time } t$

(includes free molecules and those being degraded)

$N(t) = \text{total number of protein molecules in system at time } t$
Multiclass Queue: Processing in Random Order + Reneging

Total service rate = \( \phi(n) = \min(n, L) \mu + n \gamma \)

\( n = \) total number of protein molecules in system
Steady-State Distribution

(Quasireversible Queue)

Markovian state descriptor: ordered list of the types in the queue (incl. those being processed)

Theorem (Kelly): There is a unique steady-state distribution for the “list” Markov process. The associated steady-state distribution for the total number of molecules in the system, $N$, is:

$$P(N = n) = c \frac{\Lambda^n}{\prod_{\ell=1}^{n} \phi(\ell)}$$

and conditioned on $N=n$, the stationary distribution for the molecular count process $Q$ is a binomial distribution with parameters $(n; p_1, p_2)$:

$$P(Q = (q_1, q_2)) = P(N = n) \frac{n!}{q_1! q_2!} \ p_1^{q_1} \ p_2^{q_2}$$

$$\Lambda = \sum_i \lambda_i \quad p_i = \frac{\lambda_i}{\Lambda}$$
Moments:

\[ E[Q_i] = p_i E[N] \]
\[ E[Q_i^2] = p_i (1 - p_i) E[N] + p_i^2 E[N^2] \]
\[ \text{Var}(Q_i) = p_i^2 (\text{Var}(N) - E[N]) + p_i E[N] \]
\[ E[Q_i Q_j] = p_i p_j (E[N^2] - E[N]) \quad \text{for } j \neq i \]

Correlation:

\[ r_{ij} = \frac{E[Q_i Q_j] - E[Q_i]E[Q_j]}{\sqrt{\text{Var}(Q_i)\text{Var}(Q_j)}} \]
\[ r_{ij} = \frac{F - 1}{\sqrt{(F - 1 + 1/p_i)(F - 1 + 1/p_j)}} \quad j \neq i \]

\[ F = \frac{\text{Var}(N)}{E[N]} \quad \text{Fano factor - can be computed exactly} \]
Moments for $N$

- **Distribution**: \( P(N = n) = c \frac{\Lambda^n}{\prod_{\ell=1}^{n} \phi(\ell)} \)
  
  where
  \[
  \Lambda = \sum_{i} \lambda_i \quad \phi(n) = \min(n, L) \mu + n \gamma
  \]

- **Normalizing constant** $c$:
  \[
  c^{-1} = \sum_{n=0}^{L-1} \frac{\zeta^n}{n!} + \frac{\zeta^L}{L!} M(1, \beta + 1, \delta)
  \]
  \[
  \zeta = \frac{\Lambda}{\mu + \gamma}, \quad \beta = \frac{L \mu}{\gamma} + L, \quad \delta = \frac{\Lambda}{\gamma}
  \]

- **Moment generating function**: \[
  E[e^{uN}] = c \left( \sum_{n=0}^{L-1} \frac{(e^u \zeta)^n}{n!} + \frac{(e^u \zeta)^L}{L!} M(1, \beta + 1, e^u \delta) \right)
  \]

\[M(x, y, z) = \sum_{n=0}^{\infty} \frac{(x)_n z^n}{(y)_{n,n!}}\]

confluent hypergeometric function
Moments and Correlations for $Q$ ($L=1$)

$$E[Q_i] = \frac{p_i\delta M(2, \beta + 1, \delta)}{\beta M(1, \beta, \delta)},$$

$$Var(Q_i) = \frac{2p_i^2\delta^2 M(3, \beta + 2, \delta)}{\beta(\beta + 1)M(1, \beta, \delta)} - \left(\frac{p_i\delta M(2, \beta + 1, \delta)}{\beta M(1, \beta, \delta)}\right)^2 + \frac{p_i\delta M(2, \beta + 1, \delta)}{\beta M(1, \beta, \delta)},$$

$$r_{ij} = \frac{h(\beta, \delta)}{(h(\beta, \delta) + p_i^{-1})^{1/2}(h(\beta, \delta) + p_j^{-1})^{1/2}},$$

$$\beta = (\mu/\gamma) + 1, \quad \delta = \Lambda/\gamma, \quad \Lambda = \sum_{i=1}^{m} \lambda_i,$$

$$f(\beta, \delta) = \frac{2\delta M(3, \beta + 2, \delta)}{\beta + 1} - \frac{\delta(M(2, \beta + 1, \delta))^2}{\beta M(1, \beta, \delta)},$$

$$g(\beta, \delta) = M(2, \beta + 1, \delta), \quad h(\beta, \delta) = \frac{f(\beta, \delta)}{g(\beta, \delta)},$$
Zero Dilution Limit for $L=1$

- For $\gamma \rightarrow 0$ and $\rho = \Lambda / \mu < 1$

\[
r_{ij} = \frac{1}{\left(1 + \frac{1}{p_i} \left(\frac{1}{\rho} - 1\right)\right)^{\frac{1}{2}} \left(1 + \frac{1}{p_j} \left(\frac{1}{\rho} - 1\right)\right)^{\frac{1}{2}}}
\]

Here $p_i = \lambda_i / \Lambda$, $p_j = \lambda_j / \Lambda$
Correlation Resonance (non-zero dilution)

- Correlation as a function of $\lambda_1$

Simulation parameters:

$\lambda_2 = 5 \quad \mu L = 10 \quad \gamma = .01 \quad \eta = 10^8$
Dynamics (Stochastic Simulations, $L=1$)

Balanced

$\lambda_2 = 5$, $\lambda_1 = 5$
$\mu = 10$

Underloaded

$\lambda_2 = 5$, $\lambda_1 = 1$
$\mu = 10$

Overloaded (with small reneging)

$\lambda_2 = 5$, $\lambda_1 = 8$
$\mu = 10$

R Simulation code: Ruibo Ma
Theorem (at balance: $\rho \triangleq \frac{\lambda_1 + \lambda_2}{\mu} = 1, \ \gamma = 0$)

Let $\hat{Q}_i^r(t) = \frac{Q_i(r^2t)}{r}, \ i = 1,2$ (diffusion scaling)

As $r \to \infty$,

$\hat{Q}_i^r(\cdot) \to \lambda_i \tilde{W}(\cdot), \ i = 1,2$ (convergence in distribn)

where $\tilde{W}$ is a one-dimensional reflecting Brownian motion.

$\tilde{W}(t)$
Generalizations

- Finitely many types of proteins $X_1, \ldots, X_m$

\[
\begin{align*}
\emptyset &\xrightarrow{\lambda} X_i \quad \text{(production)} \\
X_i + E &\xrightarrow{\eta} X_iE \quad \text{(binding of enzyme)} \\
X_iE &\xrightarrow{\mu} E \quad \text{(degradation)} \\
X_iE &\xrightarrow{\gamma} E, \quad X_i \xrightarrow{\gamma} \emptyset \quad \text{(dilution)}
\end{align*}
\]

Steady-state multivariate distribution factorizes:

\[
P(Q = (q_1, \ldots, q_m)) = P(N = n) \frac{n!}{q_1! \cdots q_m!} p_1^{q_1} \cdots p_m^{q_m}
\]

\[
P(N = n) = c \frac{\Lambda^n}{\prod_{\ell=1}^n \phi(\ell)}, \quad \phi(\ell) = \mu \min(\ell, L) + \ell \gamma
\]

\[
r_{ij} = \frac{F - 1}{\sqrt{(F - 1 + 1/p_i)(F - 1 + 1/p_j)}}, \quad i \neq j,
\]

$F$ – Fano factor for $N$
Generalizations

- **Reversible binding**

\[ X_i + E \xrightleftharpoons{\eta^+}{\eta^-} X_iE \]

\[ L = 1 \]

\[ m = 2 \quad \lambda_2 = 5 \quad \mu = 10 \quad \gamma = .01 \]

\[ \eta^+ = 10^8(K = 0) \quad \eta^- = 1000(K > 0) \quad K = \eta^- / \eta^+ \]
Generalizations

- **Reversible binding**
  \[ X_i + E \xrightleftharpoons[\eta^-]{\eta^+} X_iE \]

  \[ m = 2 \quad \lambda_2 = 5 \quad \mu = 10 \quad \gamma = .01 \]

  \[ \eta^+ = 10^8(K = 0) \quad \eta^- = 1000(K > 0) \quad K = \eta^-/\eta^+ \]

- **Fluctuating enzymes**
  \[ \emptyset \xrightarrow{\nu} E, \quad E \xrightarrow{\gamma} \emptyset, \quad X_iE \xrightarrow{\gamma} \emptyset \]

  \[ m = 2 \quad \lambda_2 = 5 \quad \mu = 1 \quad \gamma = .1 \quad \nu = 1 \]

  \[ \eta^+ = 200 \quad \eta^- = 1000 \]
Experiment
Queueing in a Synthetic Gene Network

- Two independently synthesized fluorescent proteins: YFP and CFP in *E Coli*
- ClpXP protease degrades LAA tagged proteins

- Tet promoter driving YFP
  - Repressible by TetR
  - Tunable by Doxycycline

- Lac/Ara promoter driving CFP
  - Activated by AraC
  - Tunable by Arabinose
Effect of Coupling on Mean:

\[ \lambda_2 = 1, \lambda_2 = 2, \lambda_2 = 3 \]

\[ \langle x_2 \rangle \]

\[ \mu = 4, \gamma = 0.02 \]
\[ L = 1, K = 0.2 \]

As \( \lambda_1 \) increases, means both \( X_1 \) and \( X_2 \) increase rapidly at the “balance” point, where

\[ \lambda_1 + \lambda_2 = \mu \]
Effect of Coupling on Mean:

Experiment: modulated doxycycline
Dynamic Modulation

Red trace: periodic influx of doxycycline
Green trace: response in level of YFP
Blue trace: response in level of CFP due to coupled degradation
Adaptive Enzymatic Processing (Theory)
Let us consider the case where again a perfect adaptation.

\[ \nu(Q) = \alpha N = \alpha \sum_{i=1}^{m} Q_i \]

If enzymes are underloaded - make less
If enzymes are overloaded - make more

\[ \emptyset \xrightarrow{\lambda_i} X_i, \quad \emptyset \xrightarrow{\nu} E, \]

\[ X_i + E \xrightarrow{\eta^+} X_iE \xrightarrow{\eta^-} E, \]

\[ X_iE \xrightarrow{\gamma} \emptyset, \quad X_i \xrightarrow{\gamma} \emptyset, \quad E \xrightarrow{\gamma} \emptyset. \]
Steady-State Distribution

Steady-state multivariate distribution factorizes and can express the steady-state correlations in terms of Fano factor $F$ for $N$:

$$r_{ij} = \frac{F - 1}{\sqrt{(F - 1 + 1/p_i)(F - 1 + 1/p_j)}}, \quad i \neq j,$$

For instant irreversible binding, $(N,L)$ is a two-dimensional birth-death process.
Correlation vs. $\lambda_1$ (with slow adaptation)

- **fixed $L=25$**
  - Correlation vs. $\lambda_1$
  - Red line: via Fano
  - Blue line: direct

- **adaptive $L$**
  - Correlation vs. $\lambda_1$

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$\nu$:
- $\lambda_1$:
  - $X_1$, $X_2$
- $\lambda_2$:
- $\gamma$, $\mu$

For small $L$, the number of enzymes in the stationary state is slightly below the exact balance level. For $L > 10^4$, the number of enzymes in the stationary state is $L/\mu$. Given by formula (11).

As in the case of adaptive enzymatic degradation described in the previous section, the negative feedback leads to transient regime for the 8-stage chain is shown in Fig. XX...

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$m = 2$, $\lambda_2 = 10$, $\mu = 1$

$\gamma = 0.01$, $\nu = 0.01N$
where the enzyme synthesis rate forms as a function of the protein counts prescribed by the mass-action equations for the deterministic enzyme. Interestingly, in spite of coupling of all number of all protein molecules in the system. Similar systems we can find the stable stationary solution of Eqs. (19)-(20) in the literature (see, for example, [10]), however only in a deterministic model, and with a single class of substrates for each of the enzymes.

In the adaptation features the multi-dimensional steady-state distribution for the protein counts in terms of that for a two-dimensional system. This is a manifestation of the perfect adaptation caused by the integral feedback via the regulated enzyme synthesis [10, 14]. Similar perfect adaptation is known to play a key role in making significant smaller or greater than $x_\text{max}$.

In the limit of large number of reactions (16)-(18) with two types of proteins and different values of $\lambda_i$, such that ($\lambda_i, Q_i$), that is the system after overload the initial conditions (see legend); (c) heat map of the asymptotic correlation for variable $\lambda_1, \lambda_2$.

Correlation for variable $\lambda_1, \lambda_2$.

- **fixed $L=25$**
  - $m = 2$, $\mu = 1$
  - $\gamma = .01$, $\nu = .01N$

- **adaptive $L$**
  - $m = 2$, $\mu = 1$
  - $\gamma = .01$, $\nu = .01N$
Effect of $\alpha$

\[ m = 2, \quad \nu = \alpha N, \quad \lambda_1 = 10, \quad \lambda_2 = 15, \quad \mu = 1, \quad \gamma = .01 \]

\[ \gamma^2 / \mu = \alpha \leq \gamma \]
Effect of $\alpha$

$m = 2, \; \nu = \alpha N, \; \lambda_1 = 10, \; \lambda_2 = 15, \; \mu = 1, \; \gamma = .01$

$\gamma^2 / \mu = \alpha \leq \gamma$
Enzymatic Networks with Shared Resources

parallel network with shared enzyme

serial network with shared enzyme

networks with shared cofactor
Conclusions

• Shared processing resources produce correlated behavior in enzymatic networks
• By mapping stochastic enzymatic models to multiclass quasireversible queues, we obtained explicit formulas for steady-state multi-variate distributions and correlations
• Correlations have a strong peak near balance point
• Slow adaptation of enzymatic resources leads to high correlations in broad regions of parameter space
• Theoretical predictions agree with experimental results for a two-component synthetic gene network
References


Factorized time-dependent distributions for certain multiclass queueing networks and an application to enzymatic processing networks, W. H. Mather, J. Hasty, L. S. Tsimring, and R. J. Williams, Queueing Systems 1-16.

THANK YOU