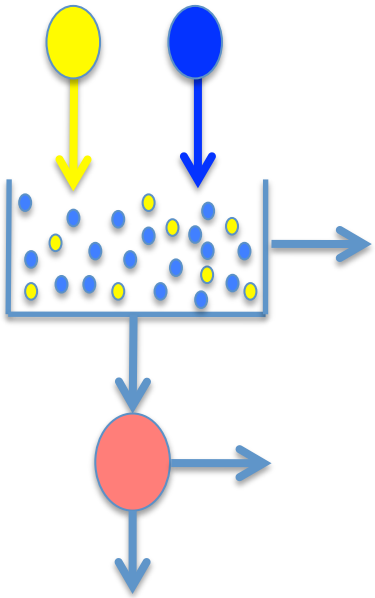
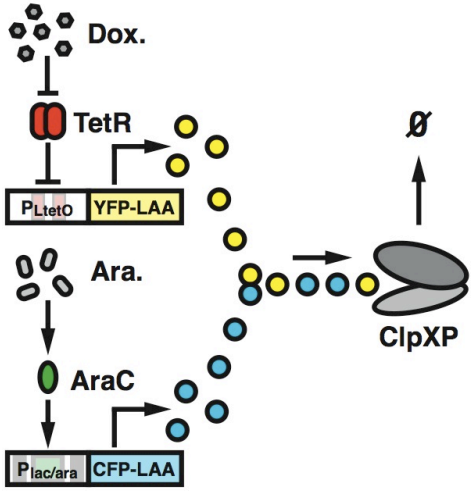
Queueing up for enzymatic processing: correlations through coupled degradation

A major challenge for systems biology is to deduce the molecular interactions that underlie correlations observed between concentrations of different intracellular molecules. Although direct explanations such as coupled transcription or direct protein-protein interactions are often considered, potential indirect sources of coupling have received much less attention. Here we show how correlations can arise generically from a posttranslational coupling mechanism involving the processing of multiple protein species by a limited number of copies of a common enzyme. By observing a connection between a stochastic model and multiclass queue, we obtain a closed form expression for the steady-state distribution of the numbers of molecules of each protein species. From analytic expressions for the moments and correlations associated with this distribution, we observe a striking phenomenon that we call correlation resonance: for small dilution rate, correlations peak near the balance point where the total rate of influx of proteins into the system is equal to the maximum processing capacity of the enzymes. The talk will describe the theoretical developments and the results of related experiments.

Based on joint work with Natalie Cookson, Tal Danino, Jeff Hasty, Will Mather, Octavio Mondragon-Palomino, Lev Tsimring.

Queueing Up for Enzymatic Processing: Correlations through Coupled Degradation





Ruth J. Williams
University of California, San Diego

Joint work with Natalie Cookson, Will Mather,
Octavio Mondragon-Palomino, Tal Danino, Jeff Hasty, Lev Tsimring

Motivation

- Measurements in gene networks (e.g., microarray data) often show correlations in levels of protein expression
- Typical explanations involve direct sources of coupling such as correlated transcription and protein-protein interactions
- Indirect coupling has not received much attention

Possible source of indirect coupling: enzymatic processing of an abundance of target molecules by a limited number of processing machines

Coupled Enzymatic Processing

Goal: To investigate the effect that processing by a common enzyme can have on correlations between numbers of proteins of different species

Thought Experiment

- Two uncoupled proteins X_1 and X_2

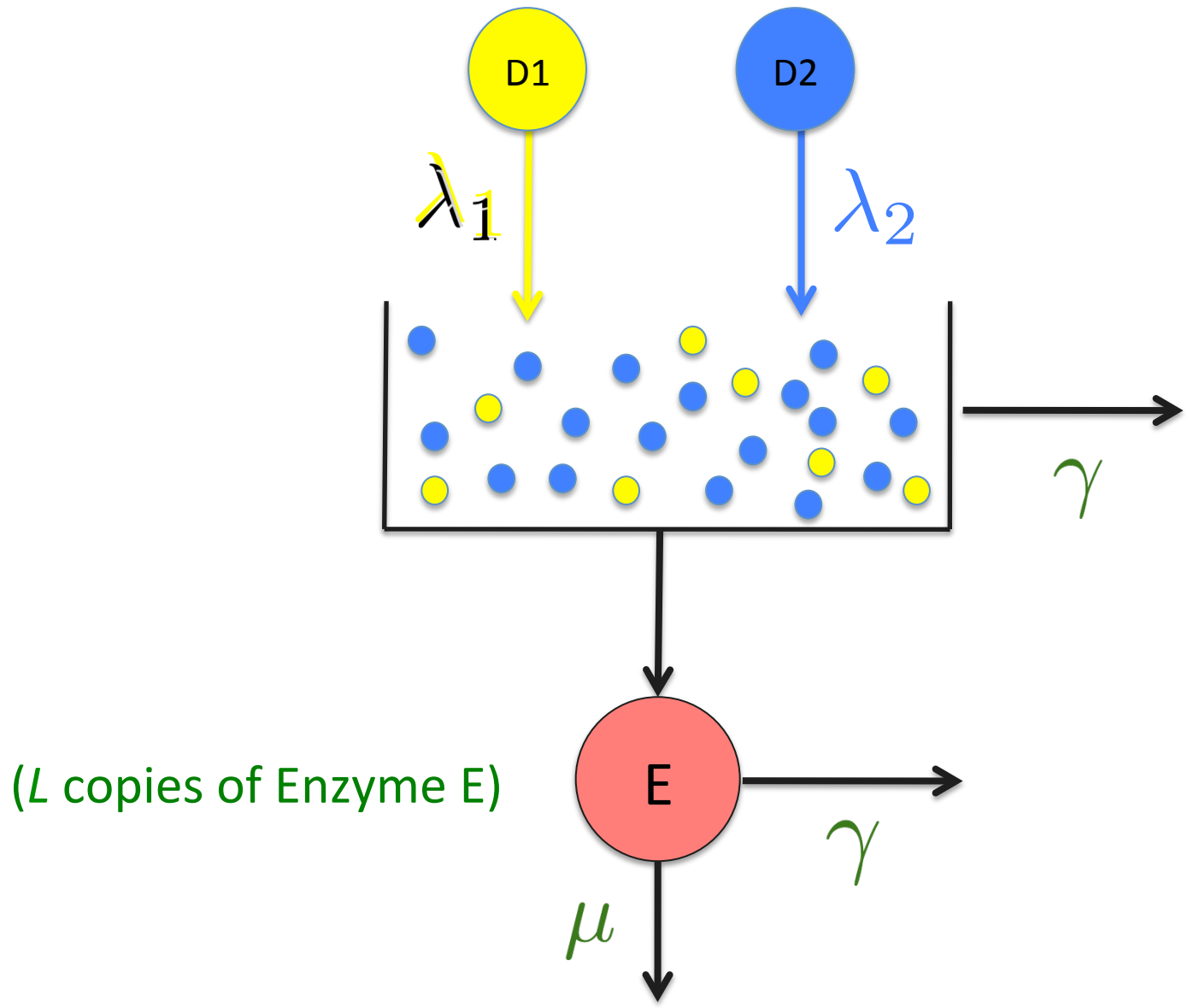
  -Processed downstream by a common enzyme *E*
- Two scenarios:

  - if *E* is abundant, X_1 and X_2 remain uncoupled

  - if *E* is limited, correlations between X_1 and X_2 appear

THEORY

Stochastic Model



Stochastic Model

Biochemical reaction network: protein species X_1, X_2

$$D_i \xrightarrow{\lambda_i} D_i + X_i \qquad \text{(production)}$$

$$X_i + E \xrightarrow{\eta} X_i E \qquad \text{(binding of enzyme)}$$

$$X_i E \xrightarrow{\mu} P_i + E \qquad \text{(degradation)}$$

$$X_i \xrightarrow{\gamma} \varnothing \quad \text{and} \quad X_i E \xrightarrow{\gamma} E \qquad \text{(dilution)}$$

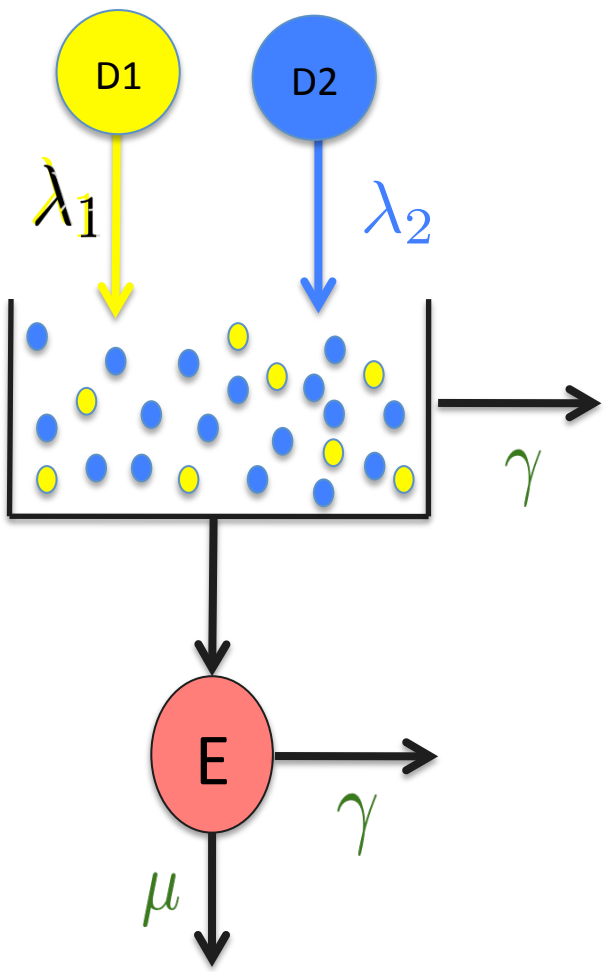
Assume: exponential reaction times and binding is instantaneous

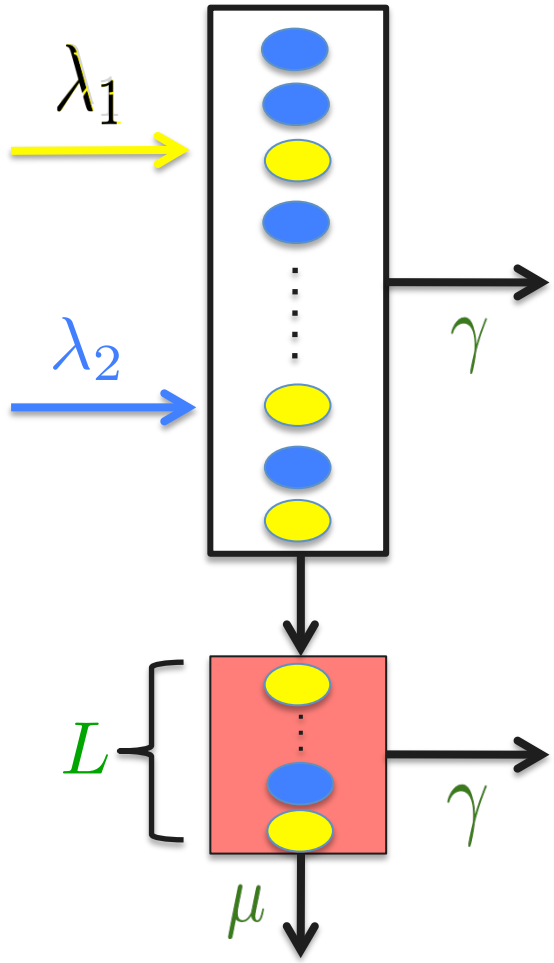
Key stochastic processes (*i=1,2*):

$Q_i(t)$ = total number of molecules of species i in the system at time t (includes free molecules and those being degraded)

$N(t)$ = total number of protein molecules in system at time t

Multiclass Queue: Processing in Random Order + Reneging





$$\text{Total service rate} = \phi(n) = min(n, L)\mu + n\gamma$$

n = total number of protein molecules in system

Stationary Distribution (Quasireversible Queue)

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

Theorem (Kelly '79): There is a unique stationary distribution for the "list" Markov process. The associated stationary 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 $(n; p_1, p_2)$:

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

where $\Lambda = \sum_i \lambda_i, \quad p_i = \frac{\lambda_i}{\Lambda}, \quad i=1,2$

Moments and Correlations

Moments:

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

Correlation ($j \neq i$):

$$
r_{ij} = \frac{\nu(N) - 1}{(\nu(N) - 1 + p_i^{-1})^{\frac{1}{2}} (\nu(N) - 1 + p_j^{-1})^{\frac{1}{2}}}
$$

$$
\text{where} \quad \nu(N) = Var(N)/E(N)
$$

Moments for *N*

- Distribution: $P(N=n) = c\dfrac{\Lambda^n}{\prod_{\ell=1}^{n}\phi(\ell)}$

  where

$$\Lambda = \sum_i \lambda_i \qquad\qquad \phi(n) = \min(n, L)\mu + n\gamma$$

- Normalizing constant *c*:

$$c^{-1} = \sum_{n=0}^{L-1}\frac{\zeta^n}{n!} + \frac{\zeta^L}{L!}M(1, \beta+1, \delta)$$

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

confluent hypergeometric function

$$\zeta = \frac{\Lambda}{\mu+\gamma}, \quad \beta = \frac{L\mu}{\gamma} + L, \quad \delta = \frac{\Lambda}{\gamma}$$

- Moment generating function:

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

Moments and Correlations for Q (L=1)

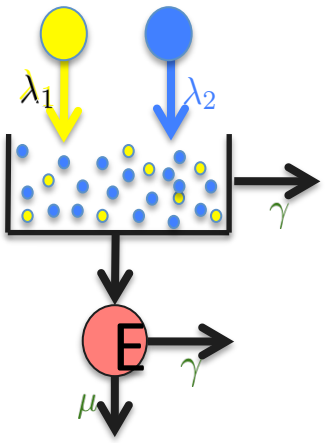
$$
\begin{aligned}
E[Q_i] &= \frac{p_i \delta M(2, \beta+1, \delta)}{\beta M(1, \beta, \delta)}, \\
Var(Q_i) &= \frac{2p_i^2 \delta^2 M(3, \beta+2, \delta)}{\beta(\beta+1) M(1, \beta, \delta)} - \left(\frac{p_i \delta M(2, \beta+1, \delta)}{\beta M(1, \beta, \delta)} \right)^2 + \frac{p_i \delta M(2, \beta+1, \delta)}{\beta M(1, \beta, \delta)}, \\
r_{ij} &= \frac{h(\beta, \delta)}{(h(\beta, \delta) + p_i^{-1})^{1/2} (h(\beta, \delta) + p_j^{-1})^{1/2}},
\end{aligned}
$$

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

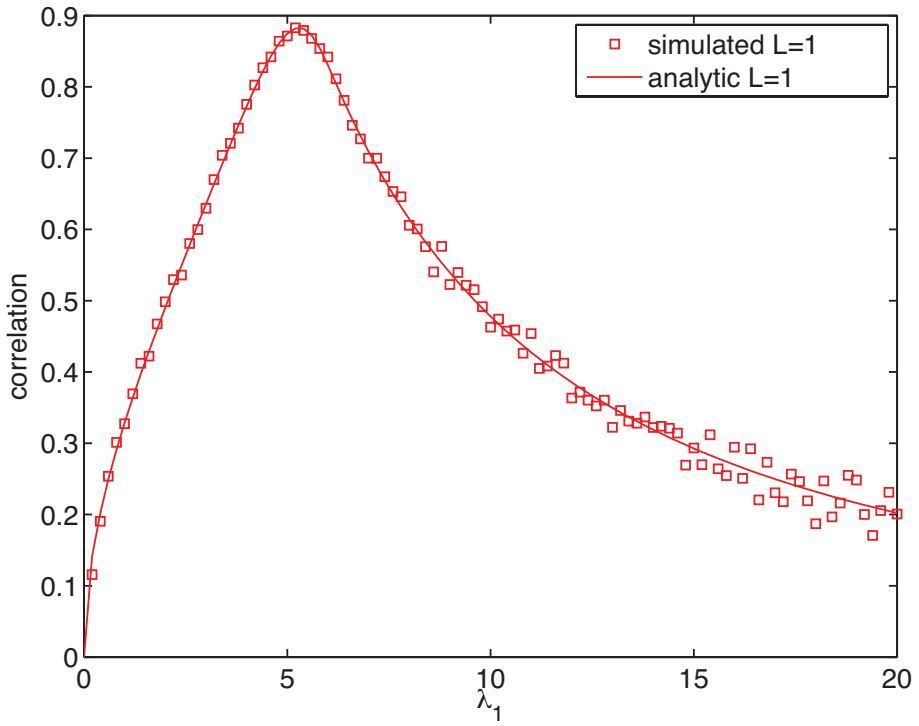
$$
f(\beta, \delta) = \frac{2\delta M(3, \beta+2, \delta)}{\beta+1} - \frac{\delta (M(2, \beta+1, \delta))^2}{\beta M(1, \beta, \delta)},
$$

$$
g(\beta, \delta) = M(2, \beta+1, \delta), \qquad h(\beta, \delta) = \frac{f(\beta, \delta)}{g(\beta, \delta)},
$$

Correlation Plots



- Plot of correlation as a function of λ_1



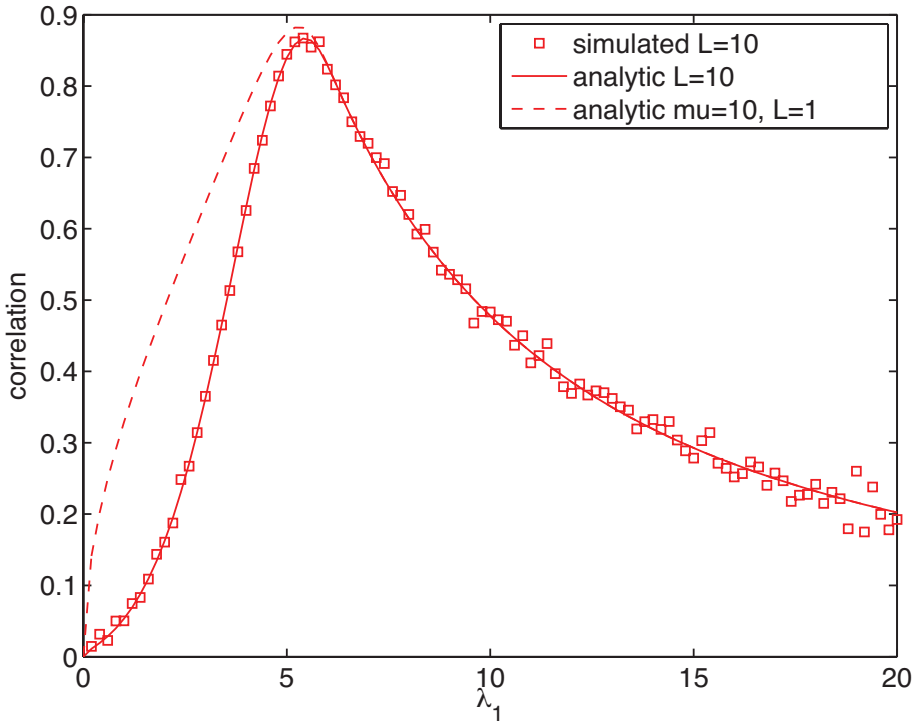
Simulation parameters:

$\lambda_2 = 5 \qquad \mu = 10$

$\eta = 10^8 \qquad \gamma = .01$

Correlation Plots

- Plot of correlation as a function of λ_1



Simulation parameters:

$\lambda_2 = 5 \qquad \mu L = 10$

$\eta = 10^8 \qquad \gamma = .01$

Zero Dilution Limit (*L*=1)

- On letting $\gamma \to 0$ for $\rho = \Lambda / \mu < 1$ and $i \neq j$:

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

Here $p_i = \lambda_i / \Lambda$, $p_j = \lambda_j / \Lambda$

Uses asymptotics from Lucy Slater's book on Confluent Hypergeometric Functions

Generalization to Reversible Binding

- Consider fast binding/unbinding reaction:

$$D_i \xrightarrow{\lambda_i} D_i + X_i \qquad \text{(production)}$$

$$X_i + E \underset{\eta_-}{\overset{\eta_+}{\rightleftharpoons}} X_i E \qquad \text{(binding of enzyme)}$$

$$X_i E \xrightarrow{\mu} P_i + E \qquad \text{(degradation)}$$

$$X_i \xrightarrow{\gamma} \varnothing \quad \text{and} \quad X_i E \xrightarrow{\gamma} E \qquad \text{(dilution)}$$

- Michaelis-Menten type approximation: replace μ by

$$\frac{n\mu}{K+n} \qquad\qquad K = \eta_-/\eta_+$$

Moments for *N*

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

  where

$$\Lambda = \sum_i \lambda_i \qquad \phi(n) = \min(n, L)\frac{n\mu}{K+n} + n\gamma$$

- Normalizing constant *c (L=1)*:

$$c^{-1} = M(\alpha, \beta, \delta)$$

$$\alpha = K+1 \qquad \beta = \frac{\mu}{\gamma} + \alpha \qquad \delta = \frac{\Lambda}{\gamma}$$

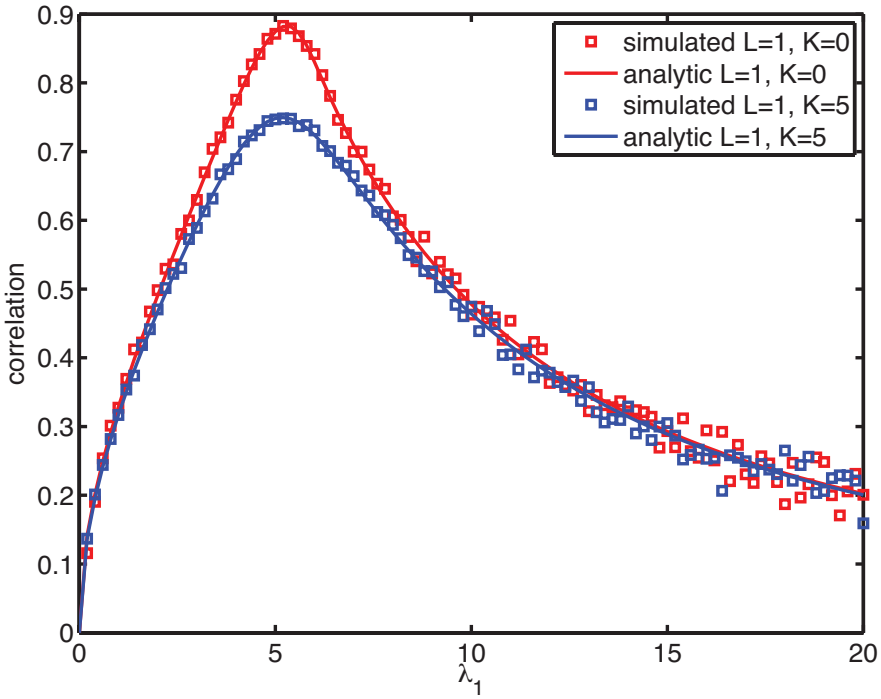
$M(x,y,z) = \sum_{n=0}^{\infty} \frac{(x)_n z^n}{(y)_n n!}$

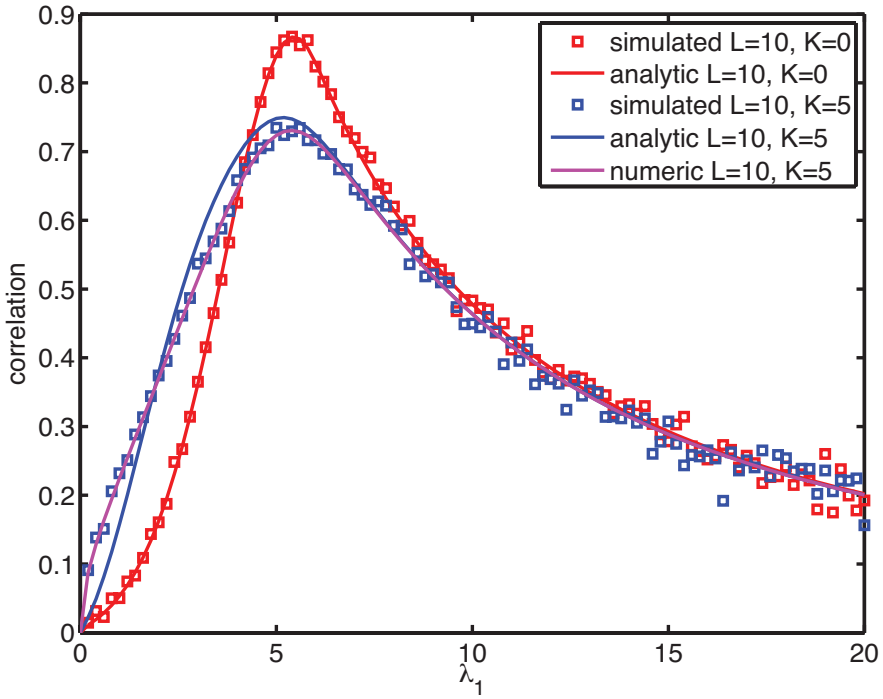
confluent hypergeometric function

- Moment generating function *(L=1)*:

$$E[e^{uN}] = cM(\alpha, \beta, e^u\delta)$$

Correlation Plots





Simulation parameters:

$\lambda_2 = 5 \quad \mu L = 10 \quad \gamma = .01$

$\eta_+ = 10^8 \quad (K = 0)$

$L\eta_- = 1000 \quad (K > 0)$

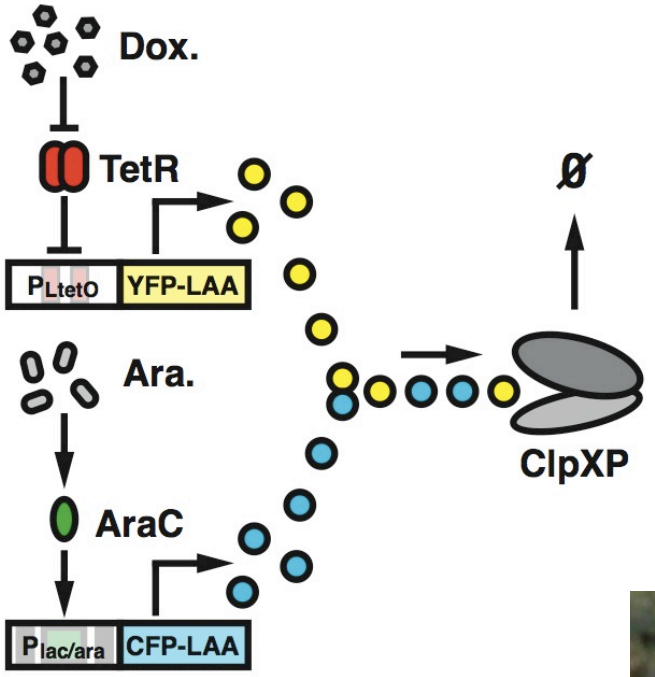
EXPERIMENT

Synthetic Genetic Network

- Enzymatic processing by *E. Coli* ClpXP machinery: targets LAA tagged proteins for degradation
- Two independently produced LAA tagged fluorescent proteins

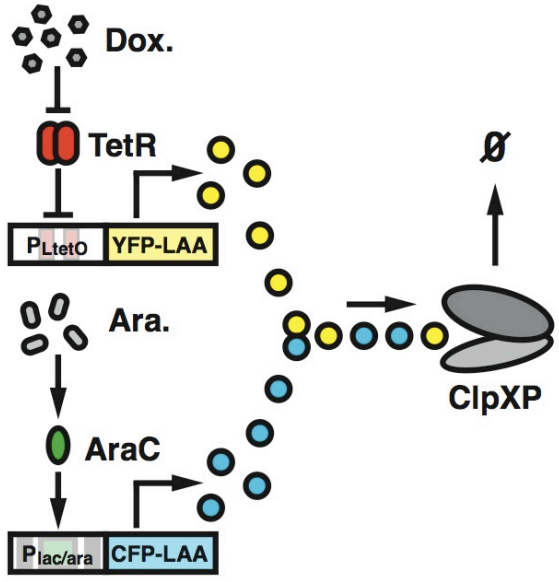
- Tet promoter driving YFP
  - Repressible by TetR
  - Tunable by Doxycycline

- Lac/Ara promoter driving CFP
  - Activated by AraC
  - Tunable by Arabinose

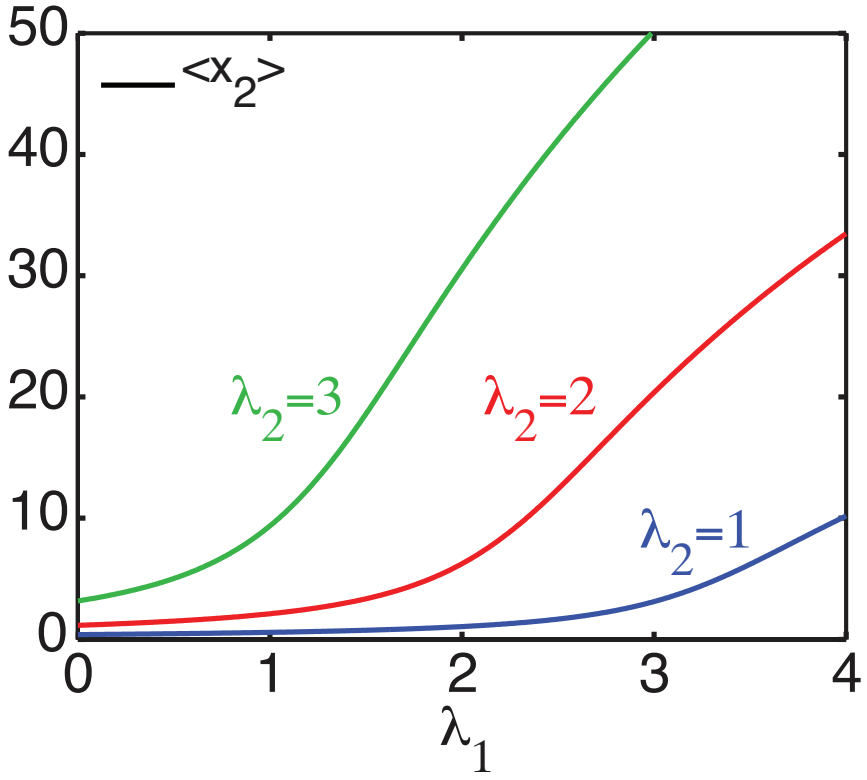


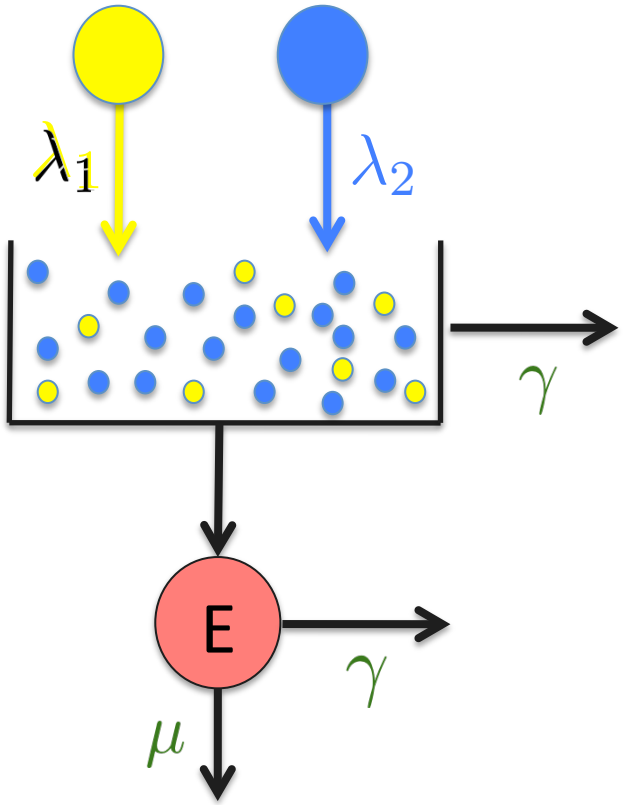
Flow Cytometry Experiment

- Theory predicts that competition for the enzymatic processors will lead to correlations in YFP and CFP levels
- Investigated this with steady-state induction data
  - Used 2-color flow cytometry to look at YFP and CFP levels
  - Modulated inducer (doxycycline) of YFP holding inducer of CFP (arabinose) constant
  - Monitored the response of both



Effect of Coupling on Mean: Theory





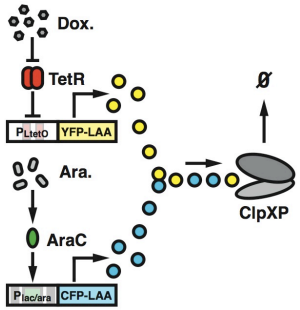
As λ_1 increases, means of X_1 and X_2 both increase and as approach "balance" point, $\lambda_1 + \lambda_2 = \mu$, have rapid increase in means.

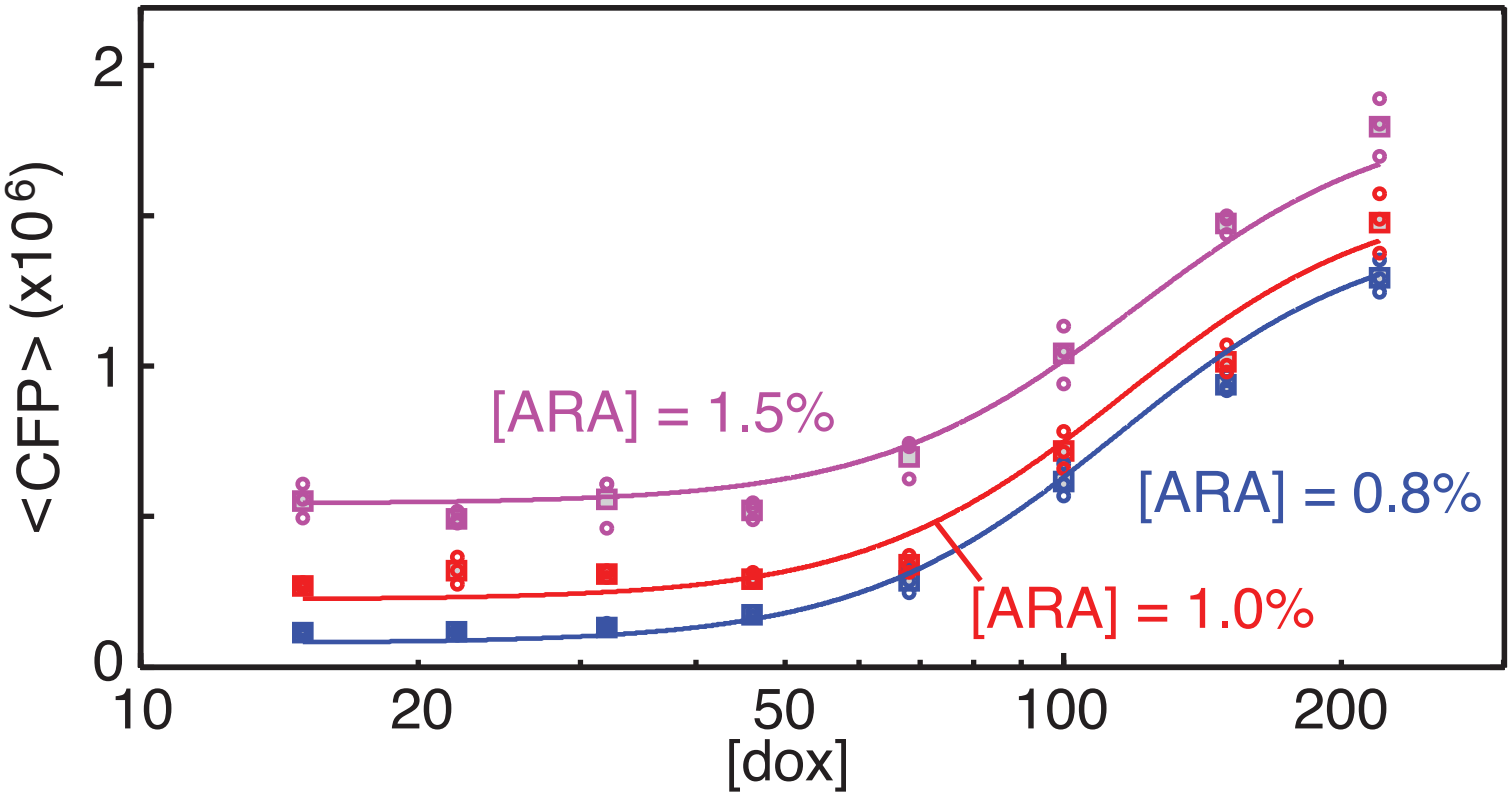
$\mu = 4 \qquad \gamma = 0.02$

$L = 1$

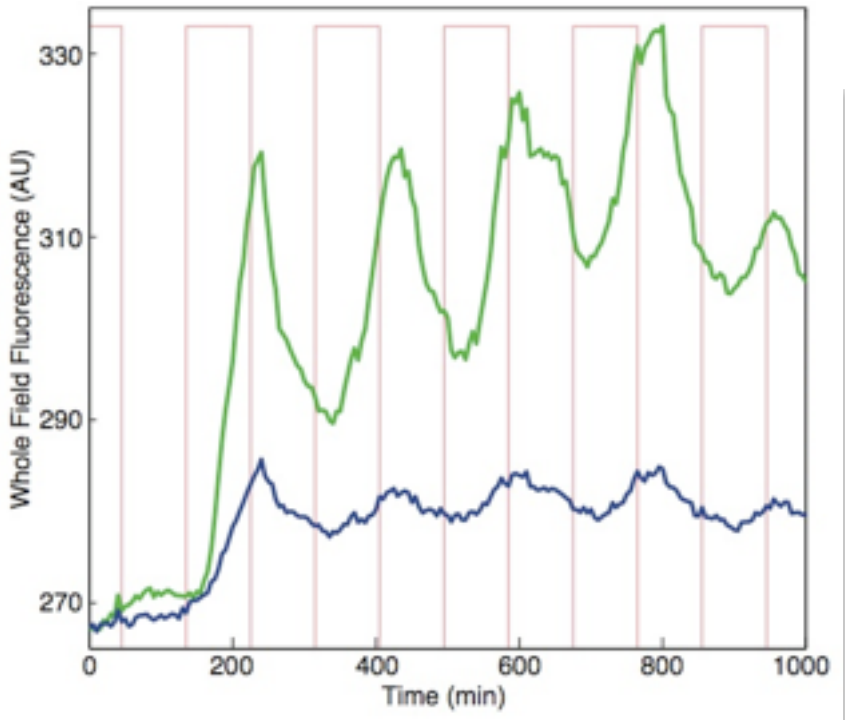
Effect of Coupling on Mean: Experiment

Modulated doxycycline





Dynamic Modulation



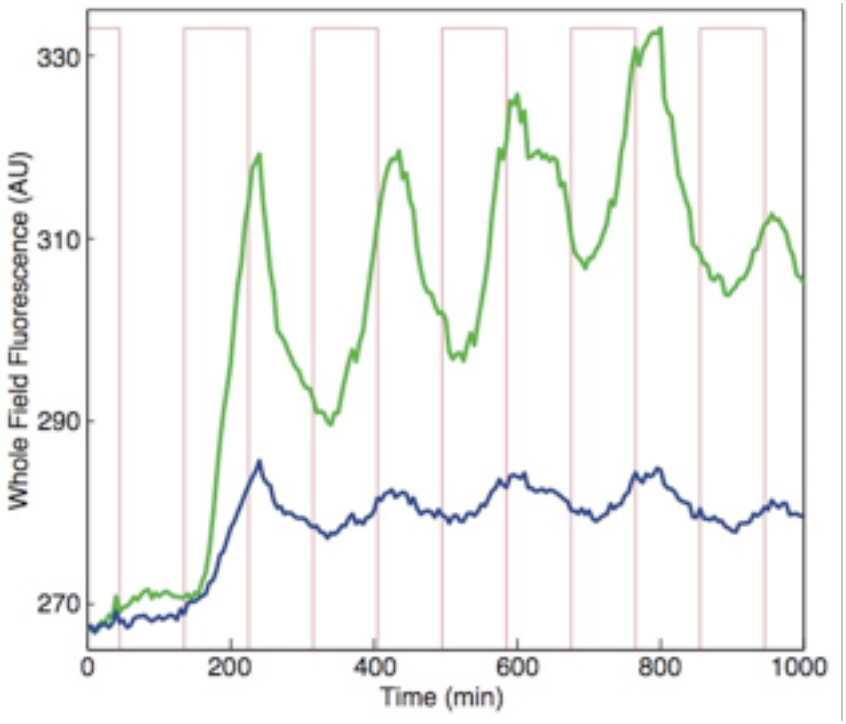
Red trace: periodic influx of doxycycline

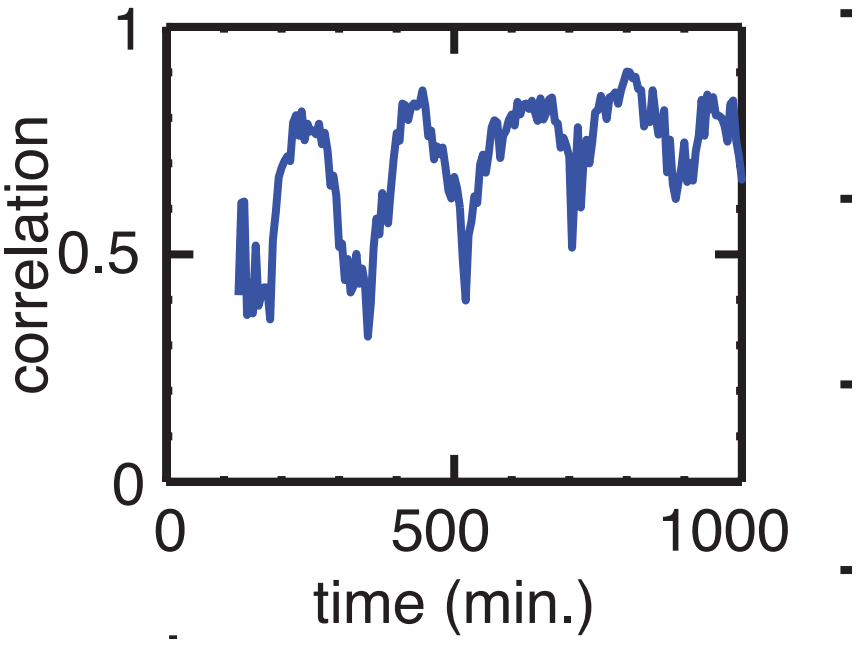
Green trace: response in level of YFP

Blue trace: response in level of CFP due to coupled degradation

Microfluidics experiment done using Dial-A-Wave and microscope

Dynamic Modulation: Correlation





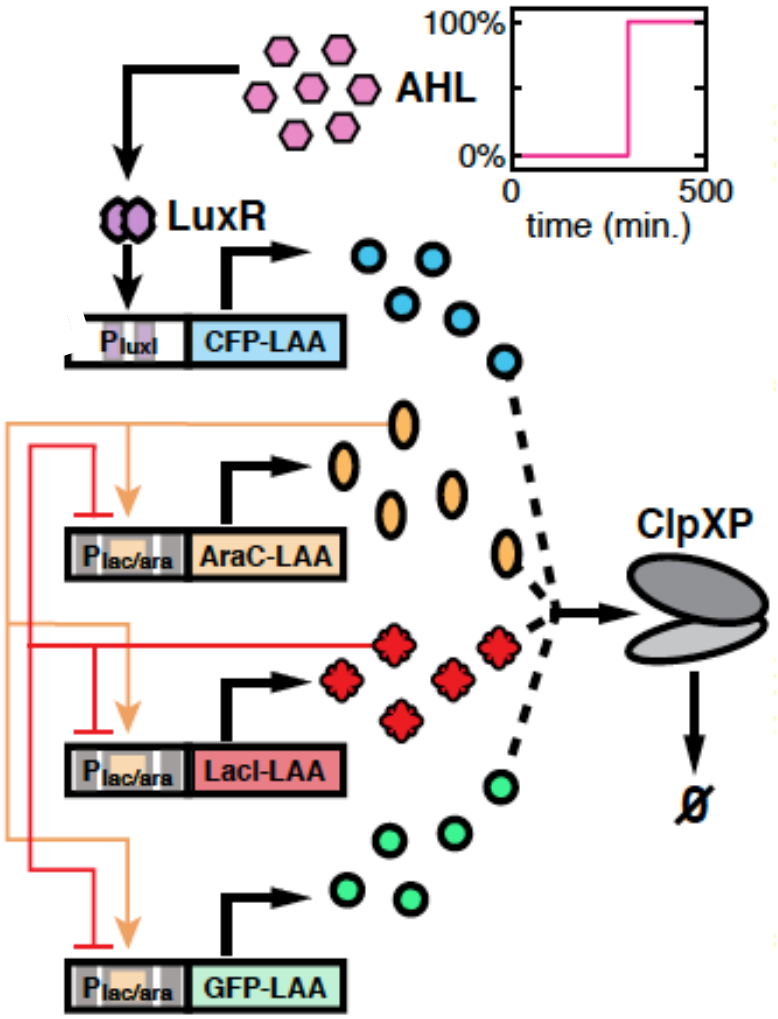
Red trace: periodic influx of doxycycline

Green trace: response in level of YFP

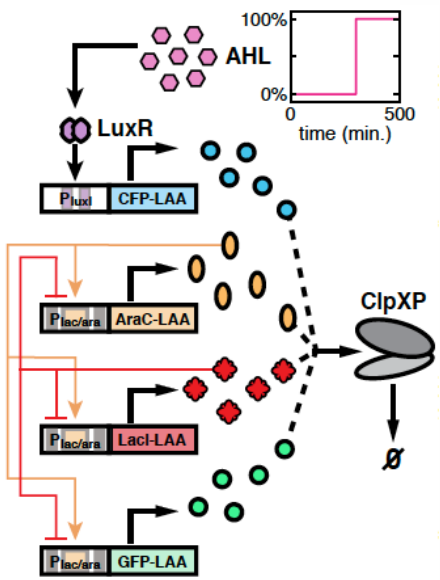
Blue trace: response in level of CFP due to coupled degradation

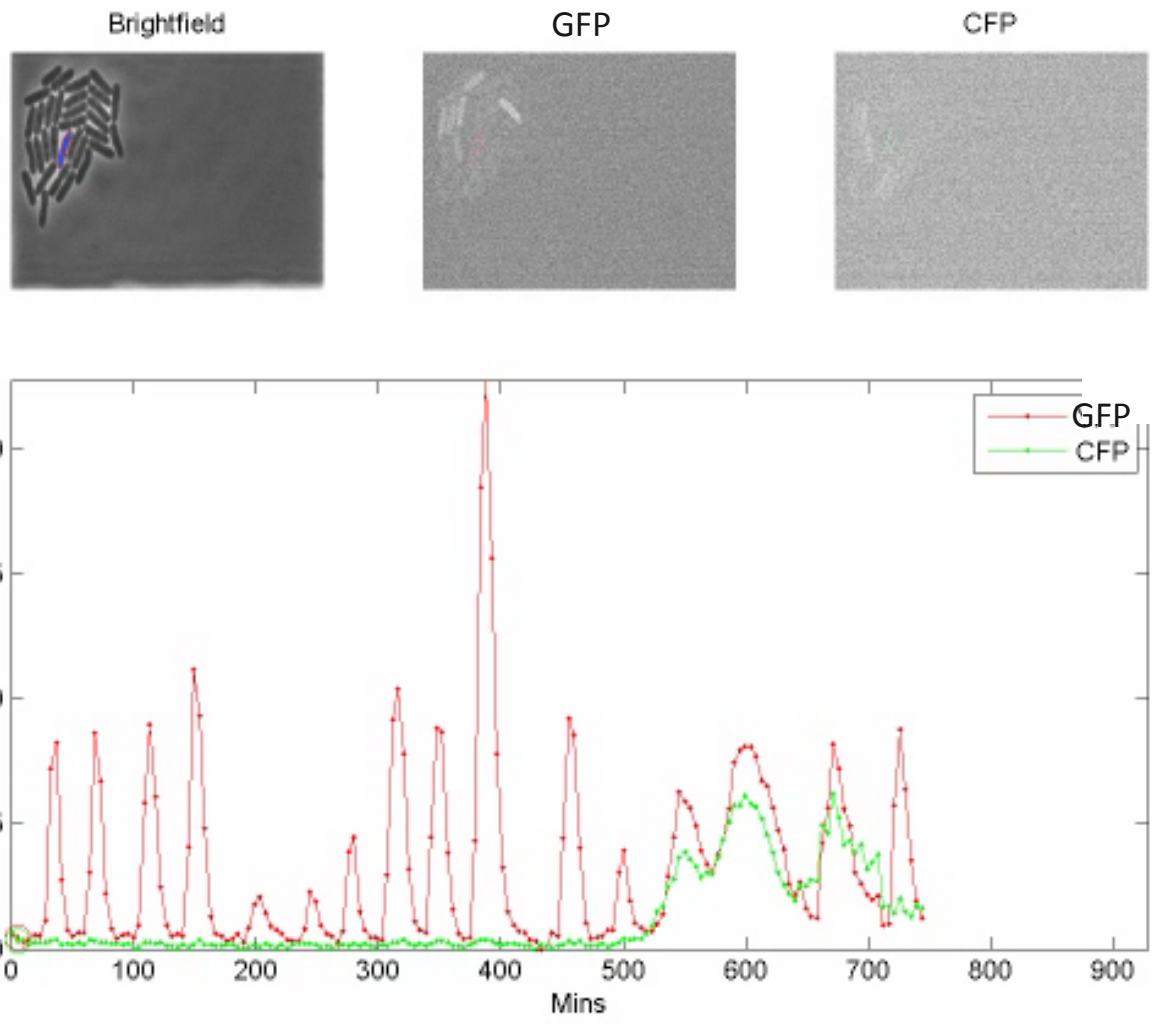
Microfluidics experiment done using Dial-A-Wave and microscope

Synthetic Oscillator with Added Competition for Degradation



Coupling with a Synthetic Oscillator



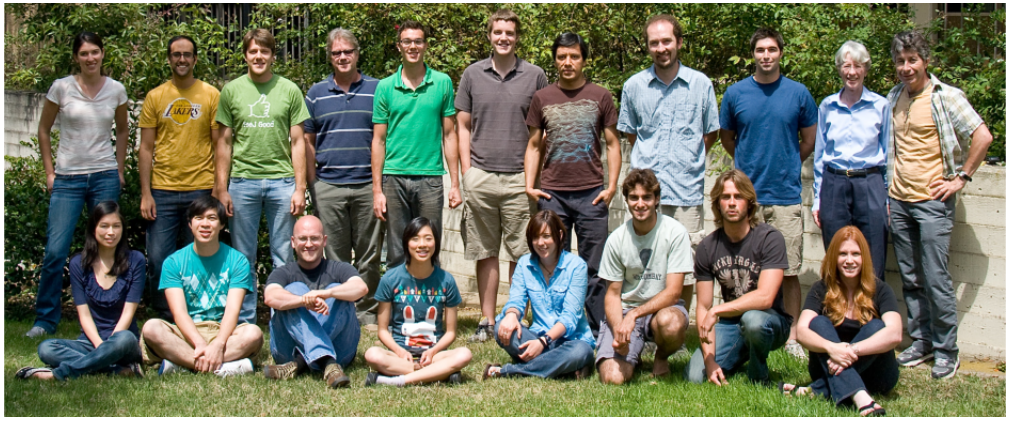


Conclusion

- Proposed a stochastic model for coupled enzymatic processing
- By mapping to a multiclass quasireversible queue, obtained stationary distribution
- Derived moments and correlations for steady-state levels of each protein
- Compared predictions with experimental results for synthetic genetic networks
- Coupled enzymatic processing produces correlated behavior that is strikingly similar to that produced by more direct sources of coupling

Acknowledgements

- Thanks to members of the UCSD Systems Biodynamics Lab.



-PIs: Jeff Hasty, Lev Tsimring

-Postdocs: Bridget Baumgartner, Natalie Cookson, Mike Ferry, Will Mather

-Graduate students: Tal Danino, Martin Kolnik, Joyce Luke, Octavio Mondragon-Palomino, Arthur Prindle, Ivan Razinkov, Phillip Samayoa, Jangir Selimkhanov, Brooks Taylor

- Thanks to NSF (IGMS), NIH, DOE, ONR for funding for this project

References

- Correlation Resonance Generated by Coupled Enzymatic Processing, W. H. Mather, N. A. Cookson, J. Hasty, L. S. Tsimring and R. J. Williams, *Biophysical Journal, 99 (2010), 3172-3181.*

- 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 (2011).

- Queueing up for enzymatic processing: correlated signaling through coupled degradation, N. A. Cookson, W. H. Mather, T. Danino, O. Mondragon-Palomino, R. J. Williams, L. S. Tsimring and J. Hasty, *Molecular Systems Biology* 7:561 (2011).

THANK YOU