Mechanical and Environmental Engineering

Design Principles in the Biological Substrate

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Thanks to
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Why Design Principles?

- Gene regulatory networks maintain vital functions.

  Failure and/or bad performance during challenges=disease/death

  Repeated failure and bad performance=replaced by a fitter specie

- Gene networks: stable, robust, responsive and reliable structures

- Random elements could have ``collapsed'' into these structures, or steered by rounds of convergent evolution to a set (possibly very large) of successful design principles.

- Latter is more likely..

- Simple argument: most successful motifs conserved by evolution, across species who evolved independently. They occur more often in real networks than in randomized networks.

N. Rosenfeld and U. Alon, Response Delays and the Structure of Transcription Networks, *J. Mol. Biol., 2003*
Savageau M.A. Genetic Regulatory Mechanisms and the Ecological Niche of *E. coli,* *PNAS, 1974*

The Focus Today…

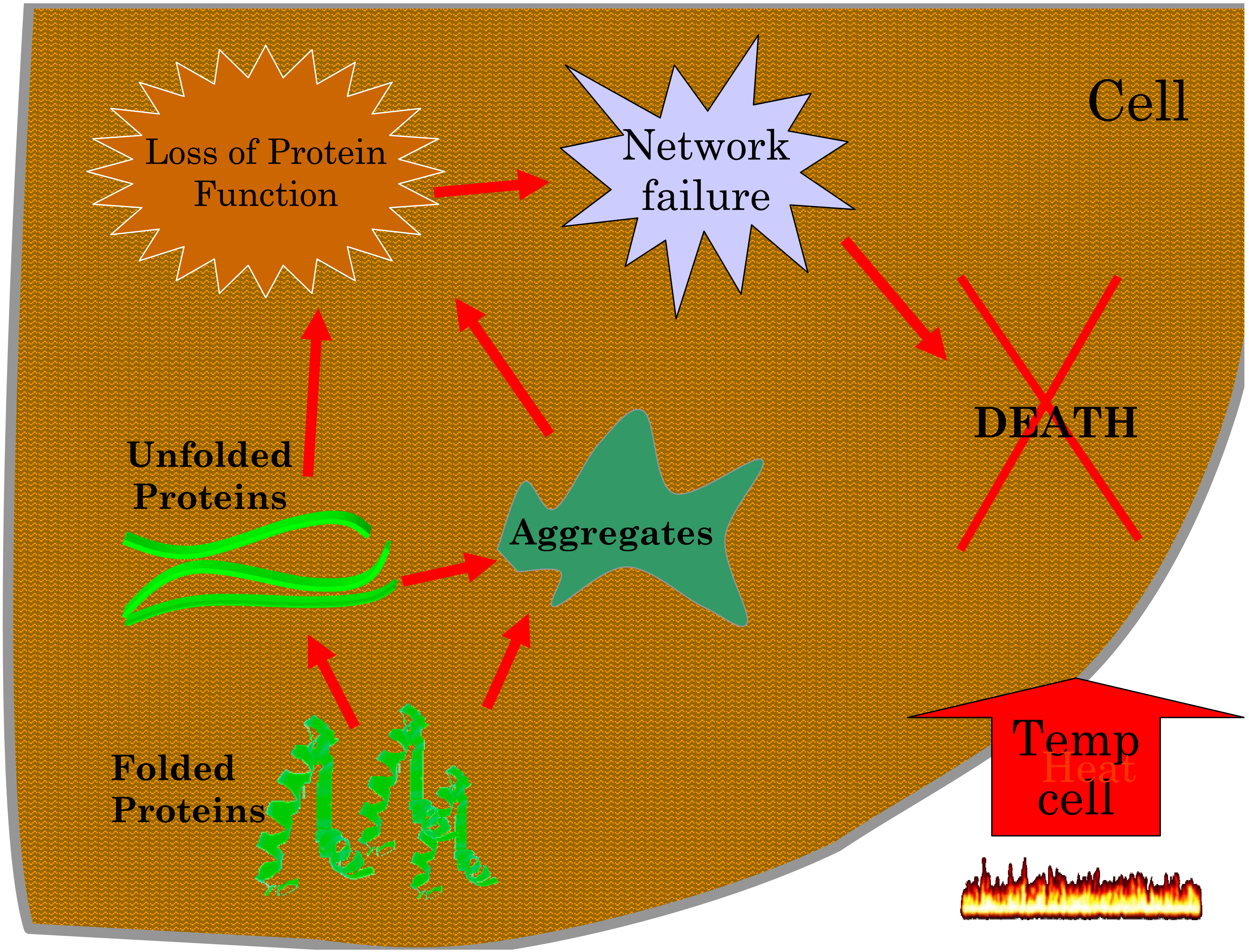
Some design principles for

- Speed, efficiency, and robustness of a typical cellular response (seen through the heat shock system).

- Stochasticity and noise attenuation (a general perspective through simple examples).

Principles of Response to Environmental Challenges

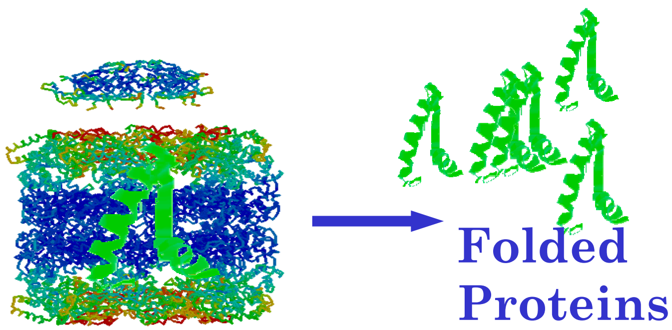
- Cells are subject to a plethora of harmful environmental conditions.
- Disrupt normal operation and exert evolutionary pressure for fast, reliable and robust defense mechanisms.
- Salient design principles in these mechanisms.
- **Heat shock response** is an excellent prototype to find a large set of these principles.



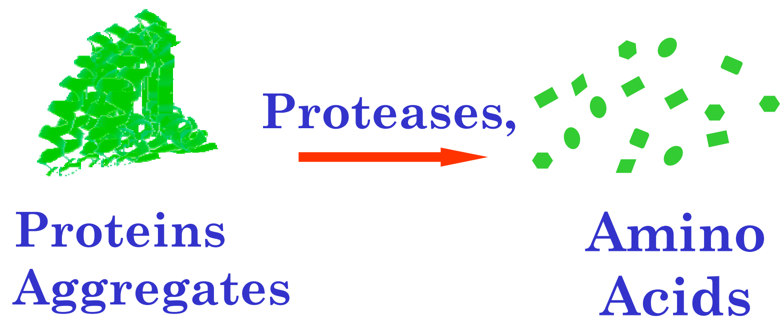
Cell
Loss of Protein Function
Network failure
DEATH
Unfolded Proteins
Aggregates
Folded Proteins
Temp
Heat
cell

Heat Shock Response

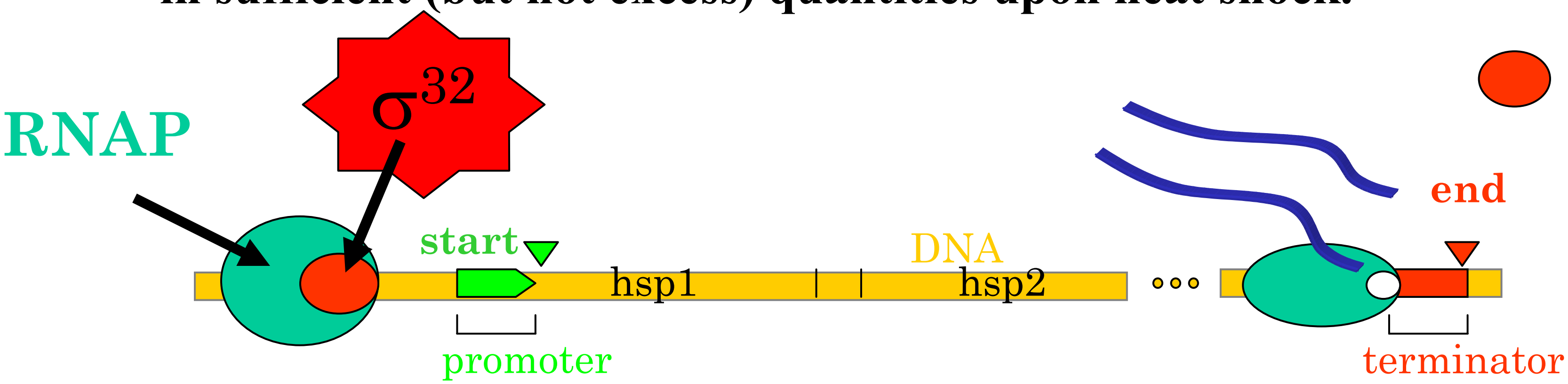
Chaperones refold unfolded proteins

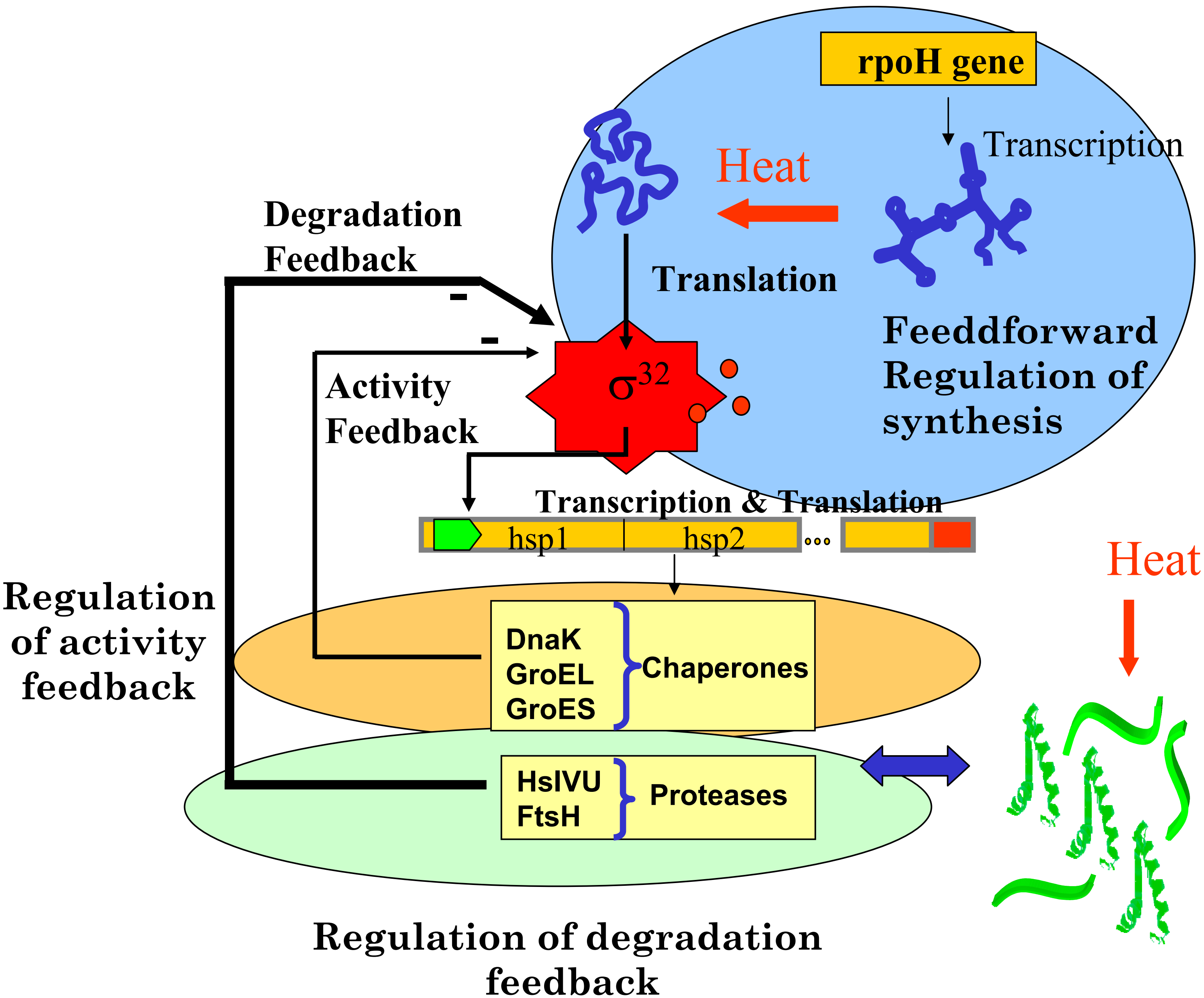


Proteases degrade aggregated proteins



Chaperones and proteases need to be produced robustly, fast, and in sufficient (but not excess) quantities upon heat shock.





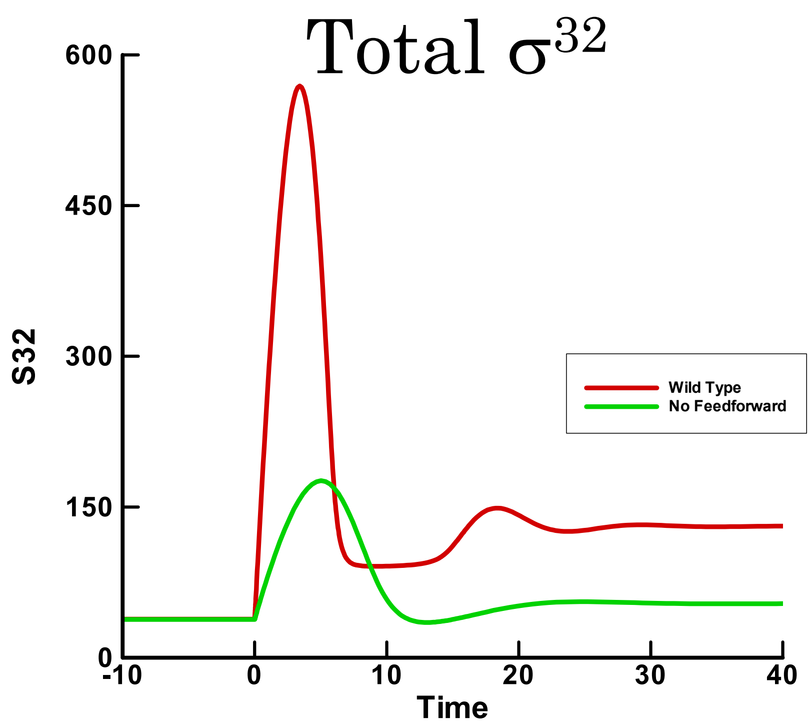
rpoH gene
Transcription
Heat
Degradation Feedback
Translation
Feeddforward Regulation of synthesis
-
-
Activity Feedback
σ32
Transcription & Translation
hsp1
hsp2
Heat
Regulation of activity feedback
DnaK
GroEL
GroES
Chaperones
HslVU
FtsH
Proteases
Regulation of degradation feedback

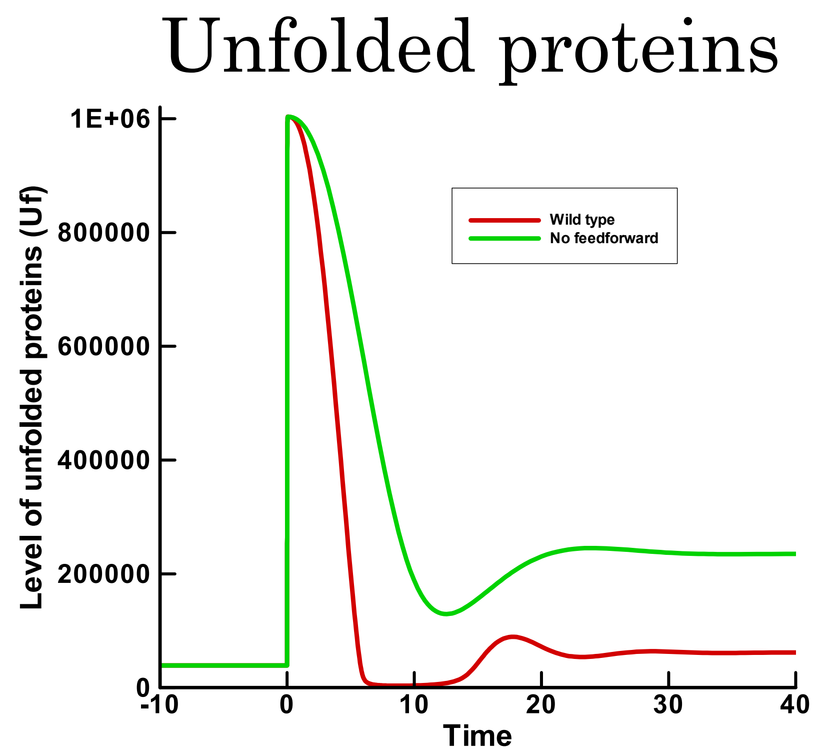
Powerful Tool

Model then Mutate

Feedforward control to achieve an efficient and fast transient response

■ **Wild Type**
■ **No Feedfoward**



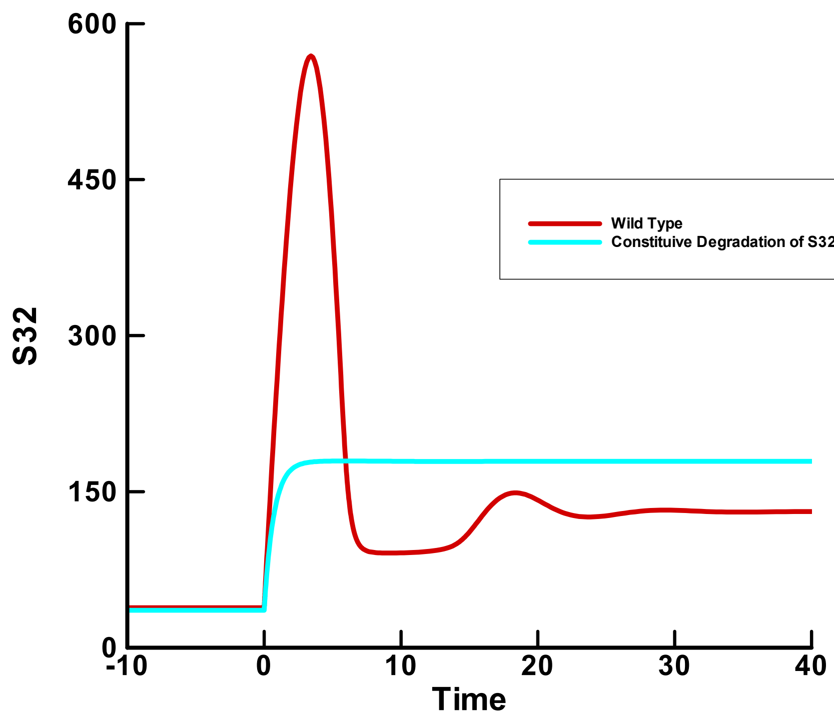


Feedback Control of Degradation of σ^{32}

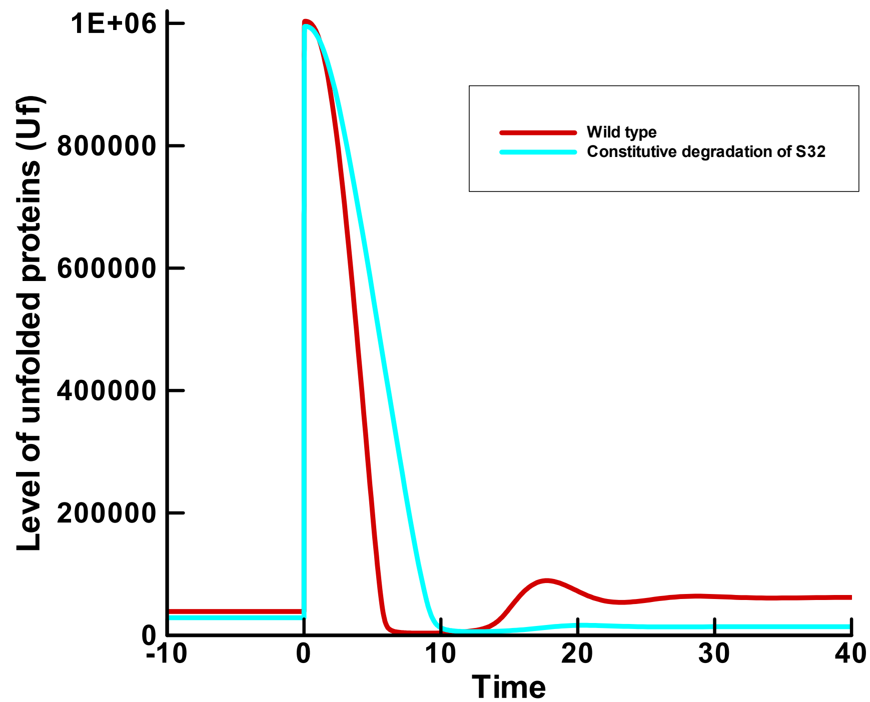
To Enhance Responsiveness

- **Wild Type**
- **Constitutive Degradation of σ^{32}**

Total σ^{32}



Unfolded proteins

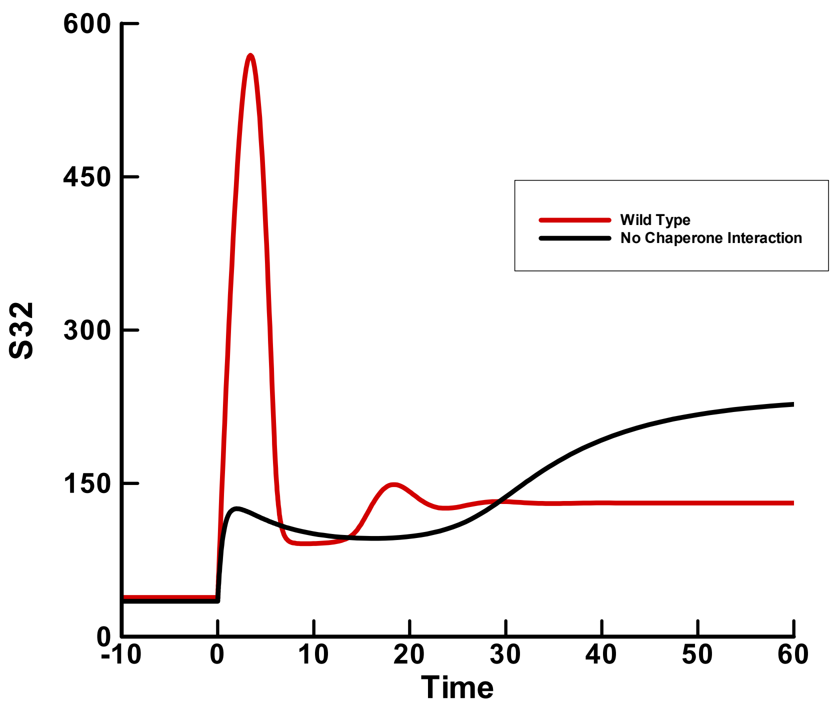


Feedback Control of Activity of σ^{32}

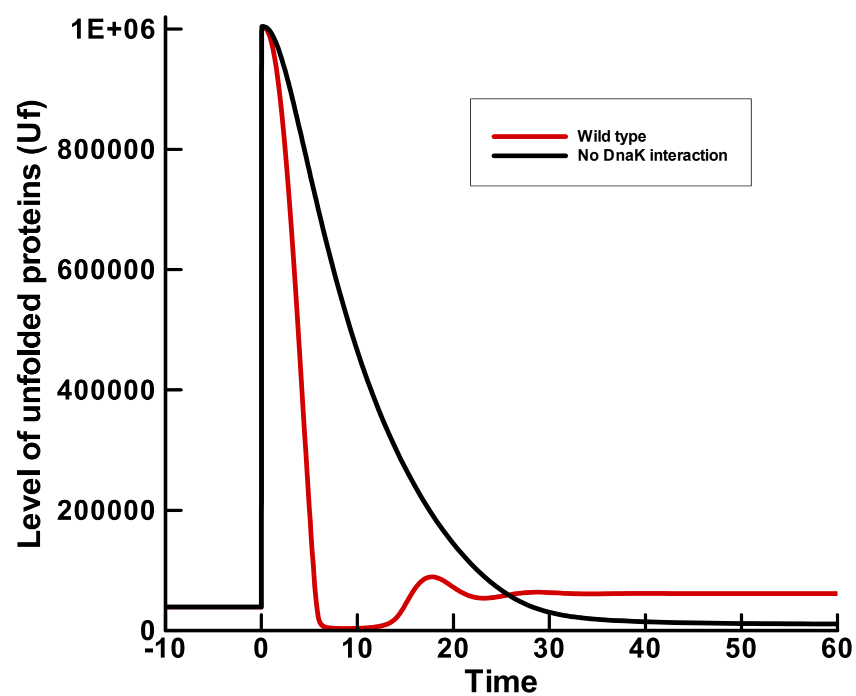
To Further Enhance Transient Response

- **Wild Type**
- **No Sequestration Loop**

Total σ^{32}



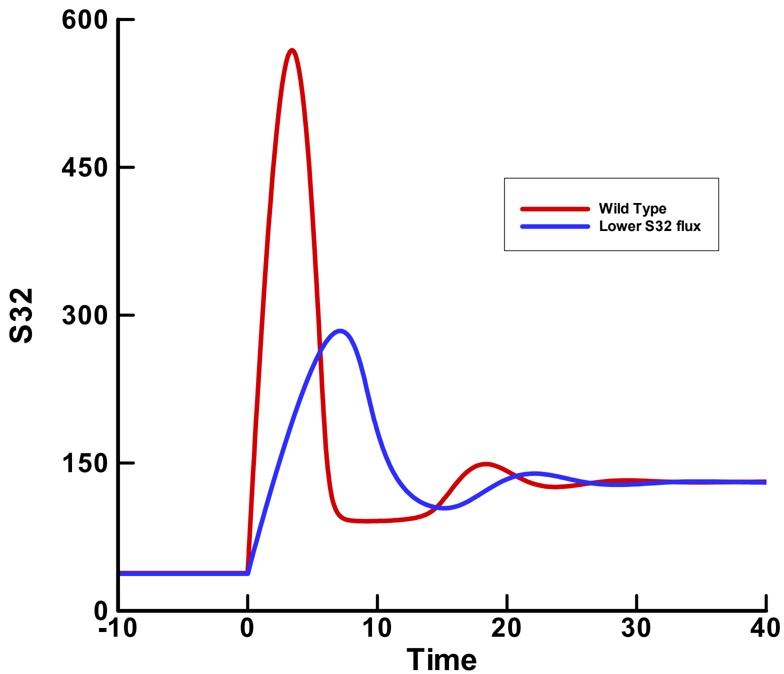
Unfolded proteins



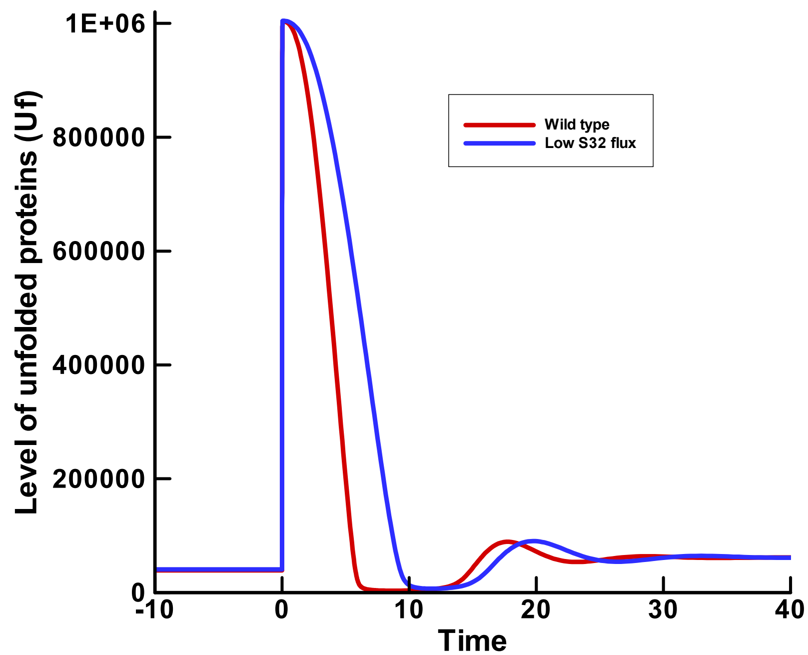
Use of High fluxes for to Enhance Transient Response

- **Wild Type**
- **Low σ^{32} Flux**

Total σ^{32}

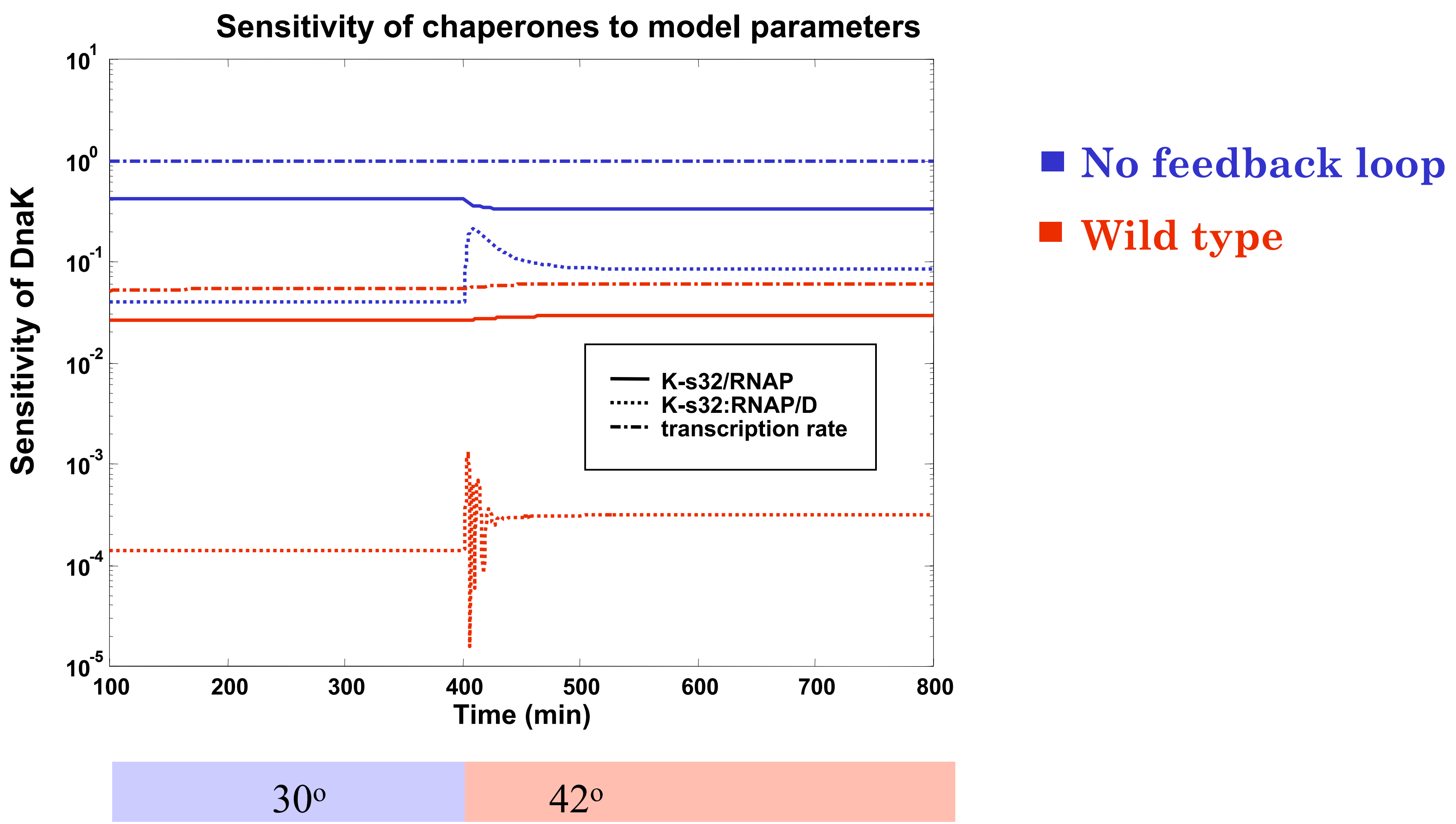


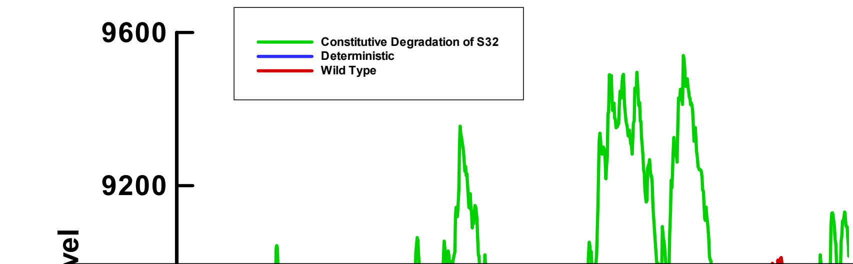
Unfolded proteins

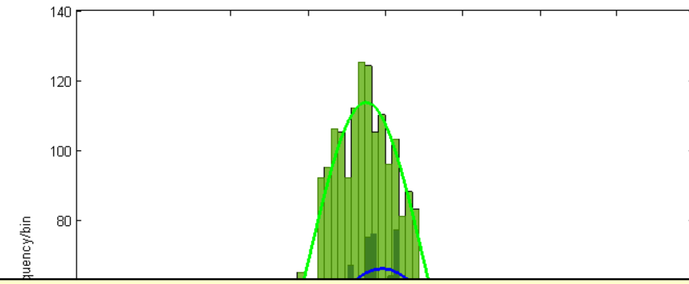


Feedback Control of σ^{32} Activity

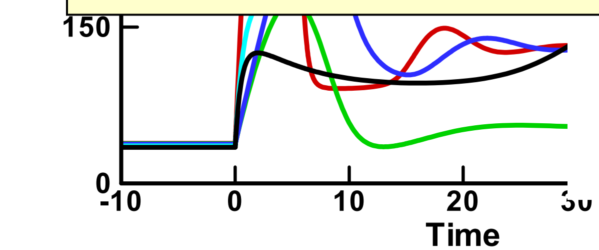
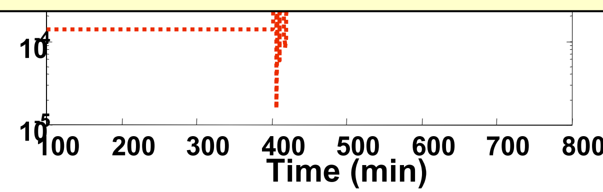
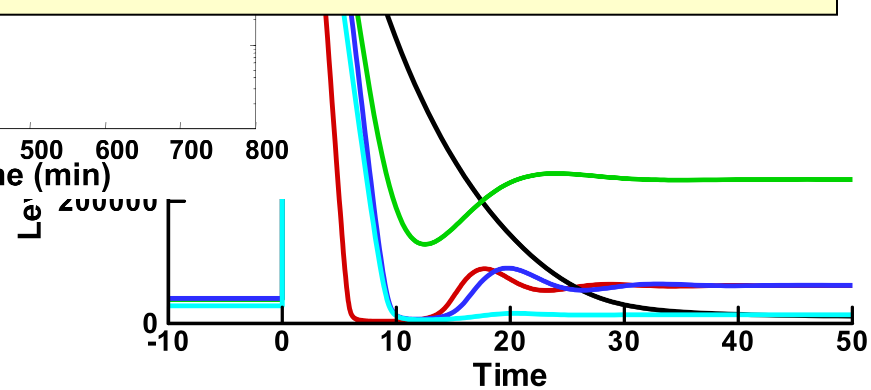
Reduction of Sensitivity to Parametric Uncertainty







- Design of the heat shock response: a necessary outcome of various performance objectives.
- Feedforward and feedback at work in a real, complex system.
- A collection of ``design principles” that were investigated in the literature in simple gene networks.

For Models of Elementary Gene Networks

Speed

Mangan, Zastaver, Alon, Use of feedforward to achieve fast OFF-ON switching, *J. Mol. Biol.* 2003

Rosenfeld, Elowitz, Alon, Use of feedback to achieve faster dynamics, *J. Mol. Biol***., 2002**

Wall, Hlavacek, Savageau, Design Principles for Regulator Gene Expression in a Repressible Gene Circuit, *J. Mol. Biol., **2003***

Schimke, Use of high fluxes to speed slow kinetics, *Curr. Top. Cell. Reg.* (1969).

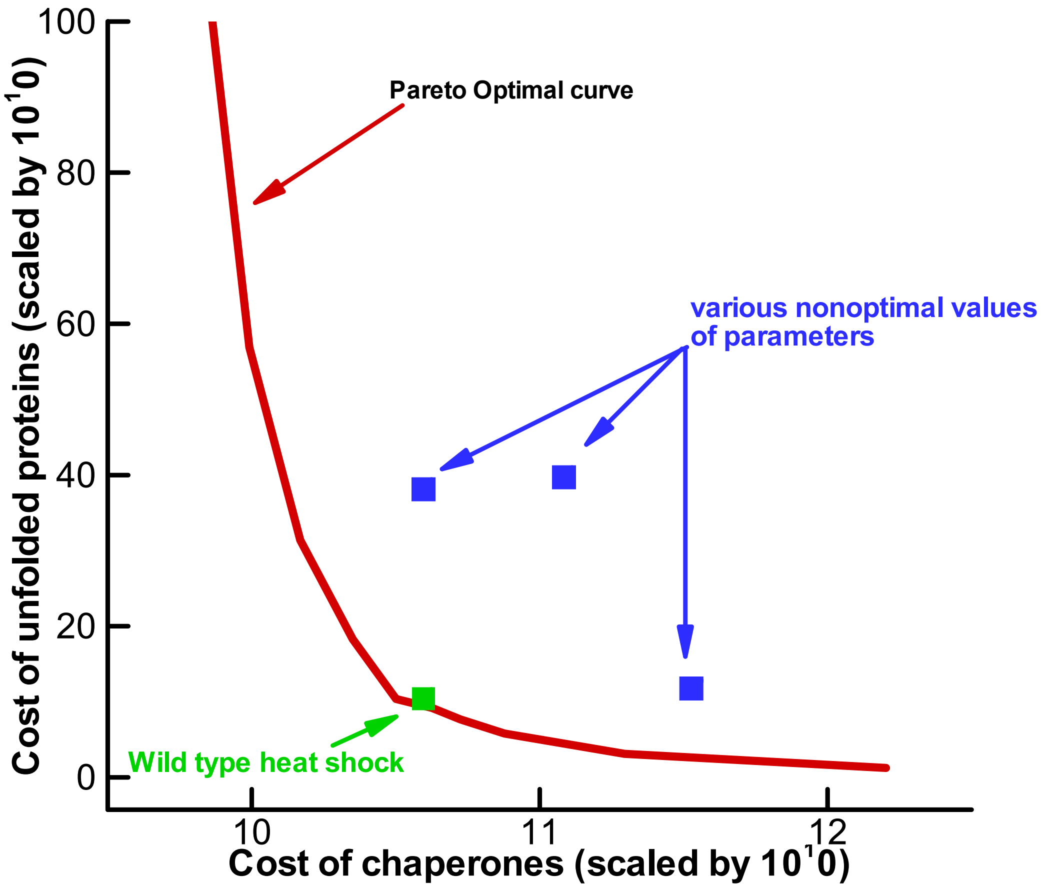
Robustness

Use of feedback to increase robustness

Ozbudack, Thattai, Kurtser, Grossman, VanOudenaarden, *Nature* (2002).

Becskei, Serrano, *Nature* (2000)

And of course, conflicting design requirements imply tradeoffs. Pareto optimality the Heat Shock System



Joint work with Chris Homescu and Linda Petzold, UCSB

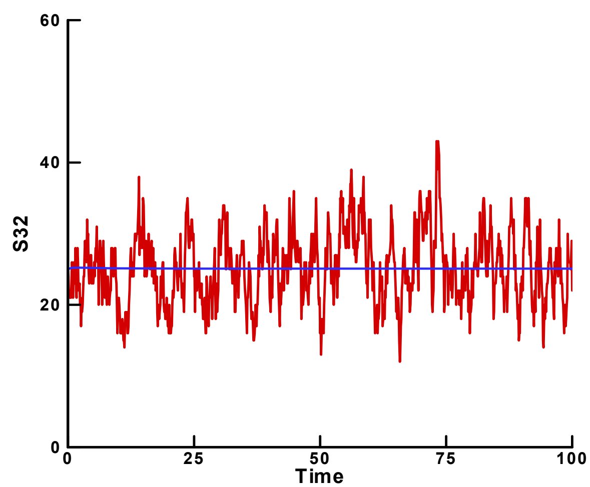
Stochasticity in Gene Networks

Fact: **Gene expression involves single molecule events, subject to thermal fluctuations. It is basically random and described by the Master Equation (ME)**

$$\frac{\partial P(x;t)}{\partial t} = \sum_{\mu=1}^{m} a_{\mu}(x-\upsilon_{\mu})P(x-\upsilon_{\mu};t) - a_{\mu}(x)P(x;t)$$

ME is not solvable

Instead, we generate ``realizations" of the system using Monte Carlo techniques (Gillespie's Stochastic Simulation Algorithm)



Stochasticity and Noise Rejection

What ``design'' features of a system define its noise rejection properties?

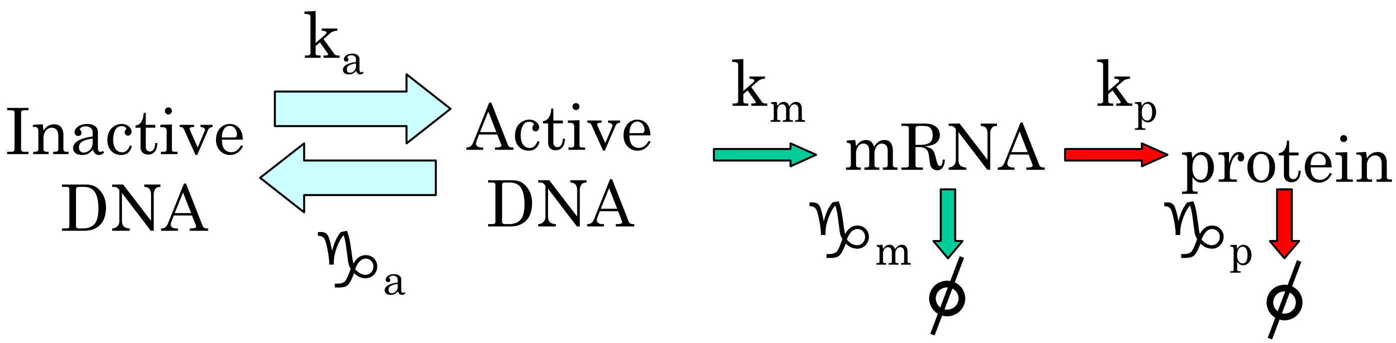
Number of Molecules	Frequency of Molecular Events	Structure and Regulation strategies
Cascade length	Stoichiometry	Etc..

•People have been working hard to establish that stochasticity is the outcome of ONLY ONE of the above…

- **Number of molecules: most cited in the literature**
- **Structure: studied a little bit**
- **Frequency of molecular events: emerging now**
- **Stoichiometry: almost never mentioned**
- **Let's see, one by one**

Frequency of Molecular Events

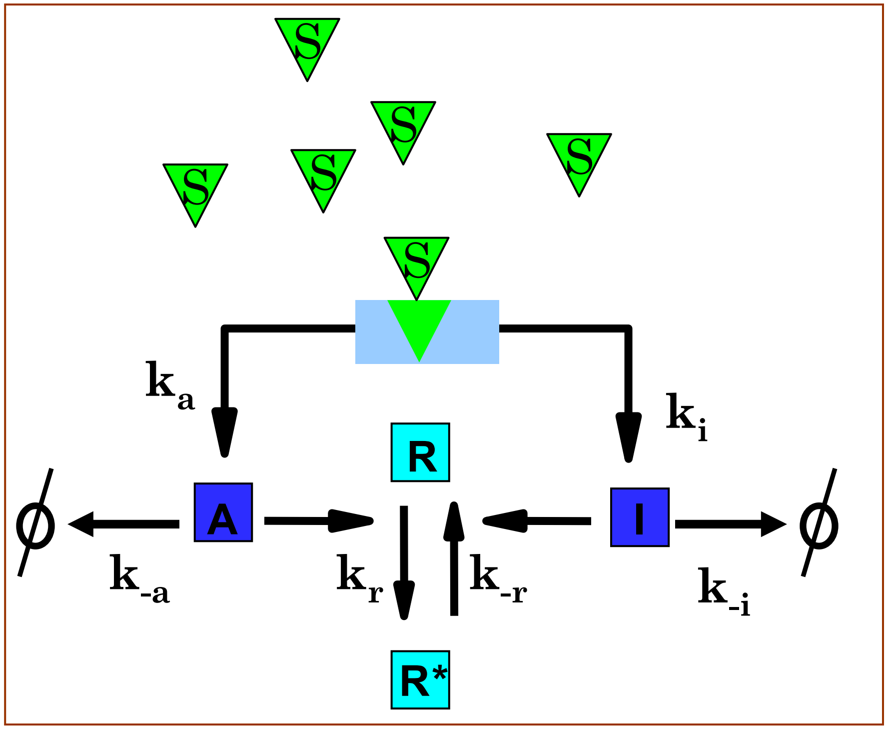
Frequent molecular events (fast reactions) constitute noise attenuation mechanisms



J. Raser and E. O'Shea, Control of stochasticity in Eukaryotic Gene Expression, *Science,* June 2004

In this system, it is true, but …

Counter Example



$$\frac{dA}{dt} = k_a S - k_{-a} A$$

$$\frac{dI}{dt} = k_i S - k_{-i} I$$

$$\frac{dR^*}{dt} = k_r A R_T - (k_{-r} I + k_r A) R^*$$

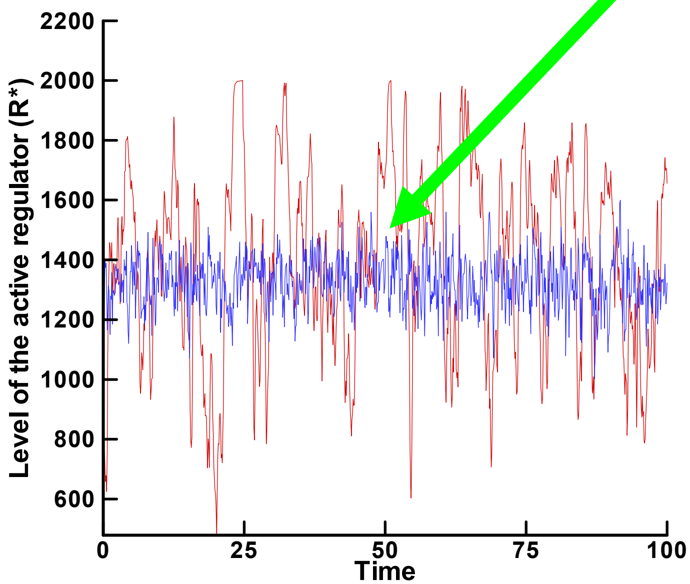
At steady state $R_{ss}^* = \dfrac{k_r R_T}{k_r + \dfrac{K_I}{K_A} k_{-r}}$ $\quad K_I = \dfrac{k_i}{k_{-i}}, K_A = \dfrac{k_a}{k_{-a}}$

P.A. Iglesias, Feedback Control in Intracellular Signaling Pathways: Regulating Chemotaxis in Dictyostelium Discodeum, *Eur. J. Control, 2003.*

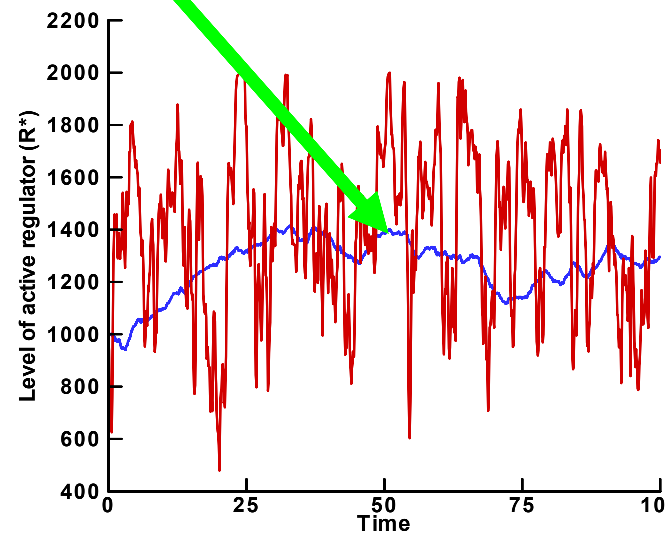
Using Linear Noise Analysis, the variance in the number of active regulators (R*)

$$\sigma_r^2 = R_{ss}^* + \frac{K_A K_I k_r^2 k_{-r}^2 R_T^2 \left(\frac{K_I}{(K_A k_r + K_I k_{-r}) S + k_{-a}} + \frac{K_A}{(K_A k_r + K_I k_{-r}) S + k_{-i}} \right)}{(K_A k_r + K_I k_{-r})^3}$$

Increase k_a and $k_{\_a}$ (or k_i and k_{-i})
(Keep ratio constant)



decrease k_r and k_{-r}
(Keep ratio constant)



Conclusion: Increasing the number of molecular reactions does not necessarily reduce fluctuations. It depends where in the network this is done.

Stoichiometry

$$nA \xrightarrow{k} X \xrightarrow{\gamma} \phi$$

n **molecules of A produce 1 molecule of X**

$$A \xrightarrow{k} nX \xrightarrow{\gamma} \phi$$

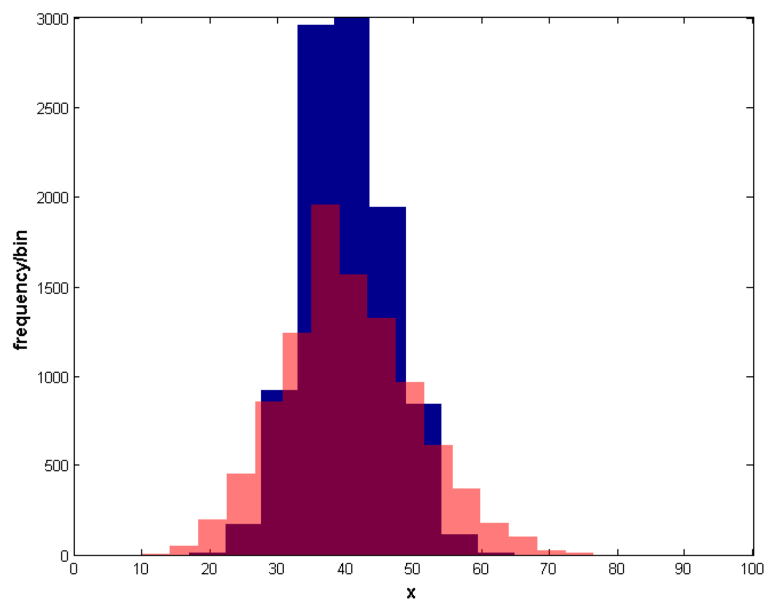
1 molecule of A produces ***n*** **molecules of X**

$$< x >= \frac{nkA}{\gamma}$$

For example, number of proteins per mRNA

Poisson

$$\text{var}(x) = \frac{nkA}{\gamma}$$

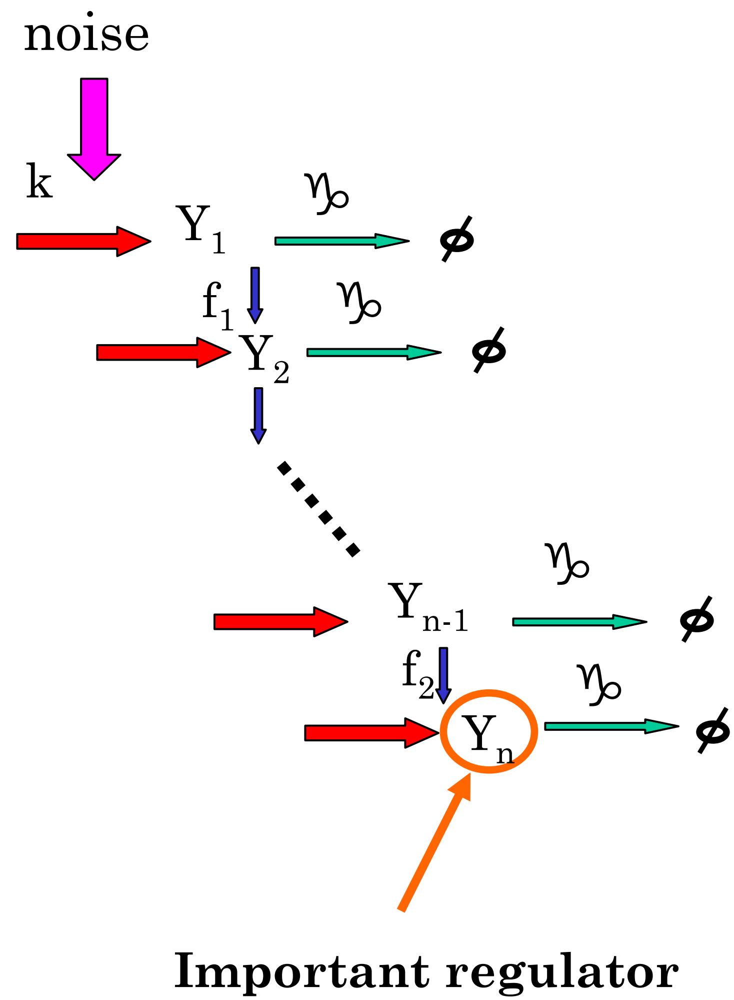


Wider than Poisson

$$\text{var}(x) = \left(\frac{nkA}{\gamma}\right).\left(\frac{n+1}{2}\right)$$

Cascade Length, Structure and coupling

- **Cascades are ubiquitous, especially in signal transduction, phosphorylation cascades, chemotaxis, MAP kinase cascades, etc..**
- **Chain of of coupled chemical reactions, with input signaling noise, and intrinsic noise at each stage.**
- **Common belief: each stage is noisier than the previous one, due to the propagation of noise.**
- **Fact: Not Necessarily true. Many ''design'' opportunities to achieve noise attenuation.**



Cascade Length, Structure and coupling

$$\sigma_n^2 \approx \frac{q}{2}\left(1+\sum_{j=1}^{n-1}\frac{c^{2j}}{\sqrt{\pi j}}\right)+\frac{q_0}{2}\left(\frac{c^{2n}}{\sqrt{\pi n}}\right)$$

Cascade noise | **Input noise**

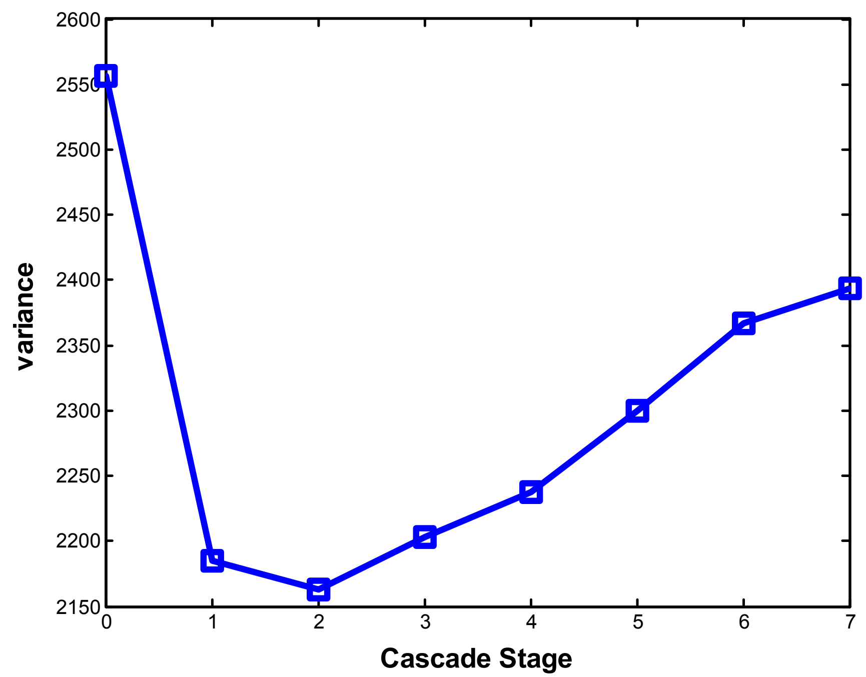
Variance at stage *n*

Cascade length

Intrinsic Noise at each stage

$c = f' < 1$

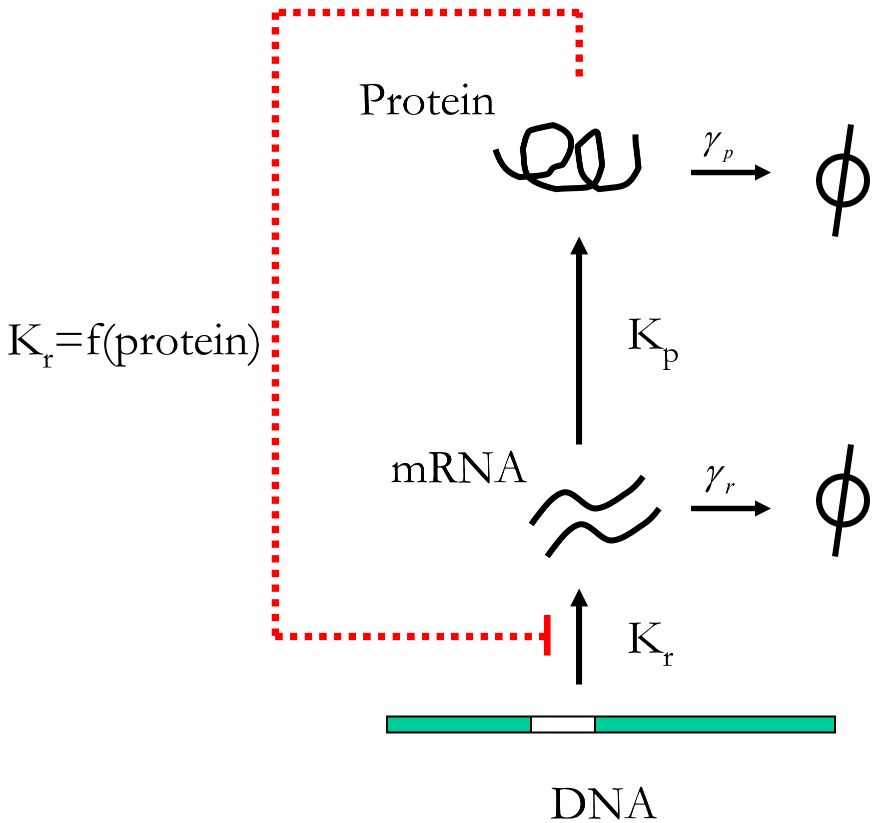
Coupling between stages



M. Thattai and A. Van Oudenaarden, Attenuation of Noise in Ultrasensitive Signaling Cascades, *Biophys. J.*, *2002.*

Regulation Strategies

Use of Feedback to Attenuate Stochastic Fluctuations



$$\eta = \frac{\gamma_p}{\gamma_r} \qquad b = \frac{K_p}{\gamma_r} \qquad \phi = \frac{K_1}{\gamma_p}$$

Open Loop

$$< p > = \frac{K_r b}{\gamma_r} \qquad \frac{\sigma^2_p}{<p>} = \left(\frac{b}{1+\eta}\right)+1$$

With proportional Feedback

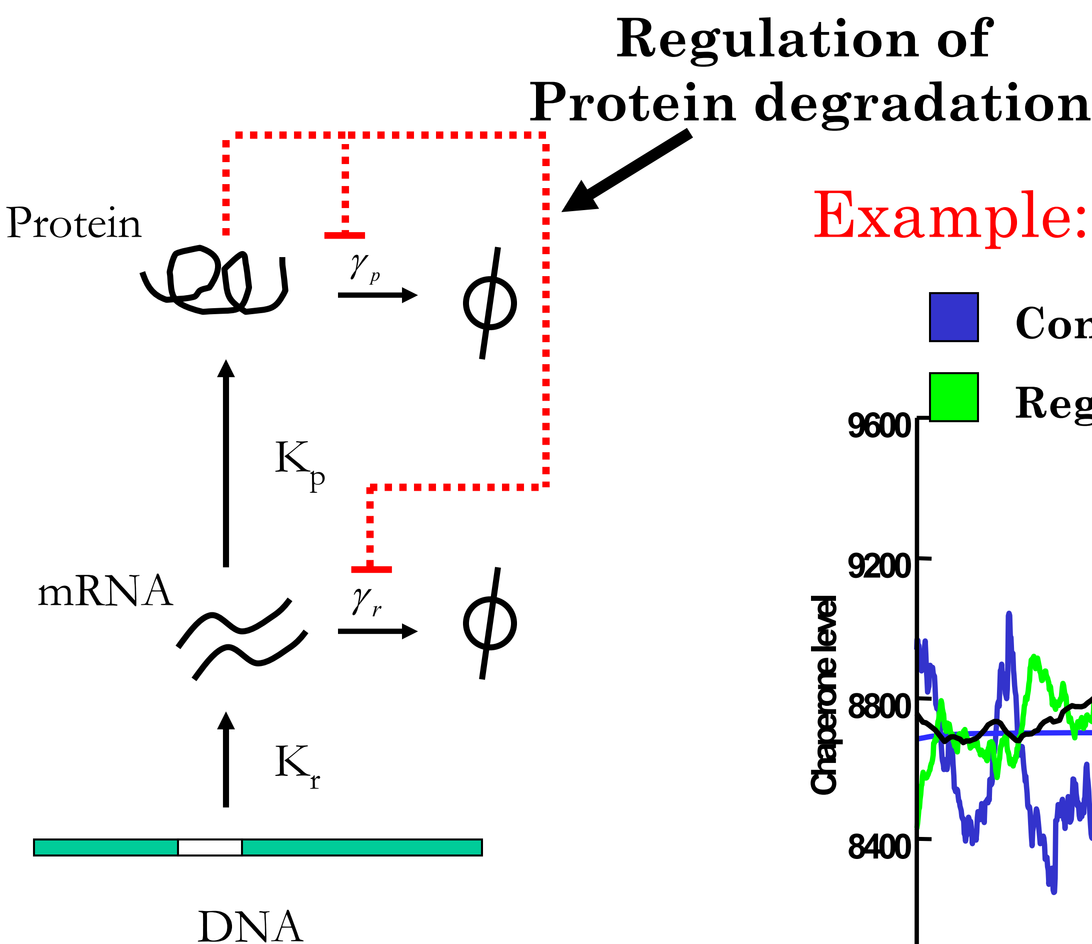
$$K_r = K_0 - K_1 p$$

$$< p > = \left(\frac{1}{1+b\phi}\right)\frac{K_0 b}{\gamma_p}$$

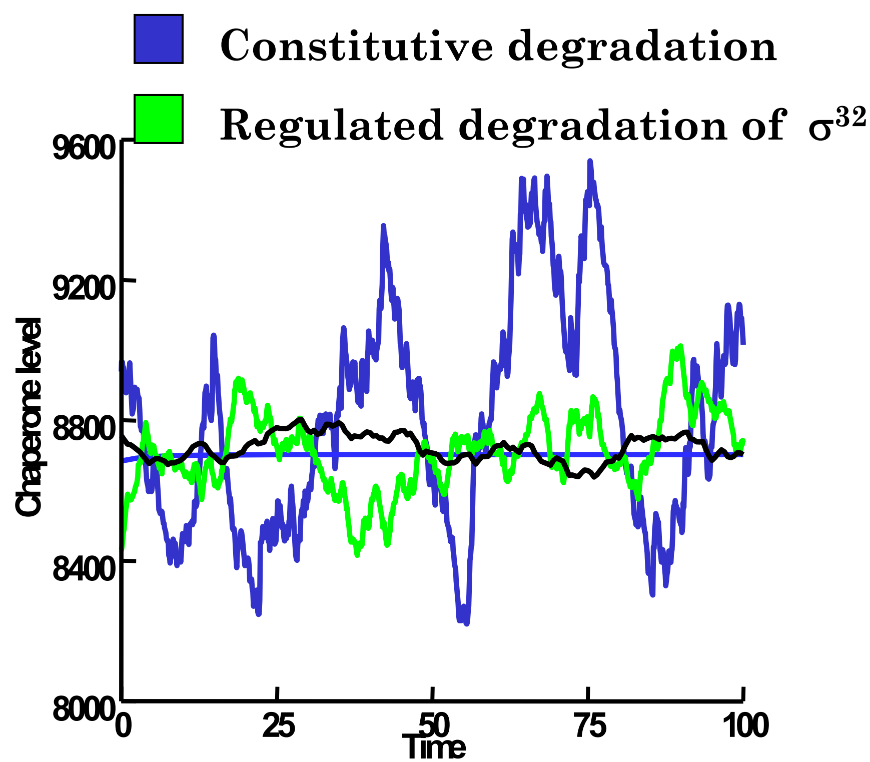
$$\frac{\sigma^2_p}{<p>} = \left(\frac{1-\phi}{1+b\phi}\right)\left(\frac{b}{1+\eta}\right)+1$$

M. Thattai, A. Van Oudenaarden, ***Intrinsic Noise in Gene Regulatory Networks,*** **PNAS, 2001**

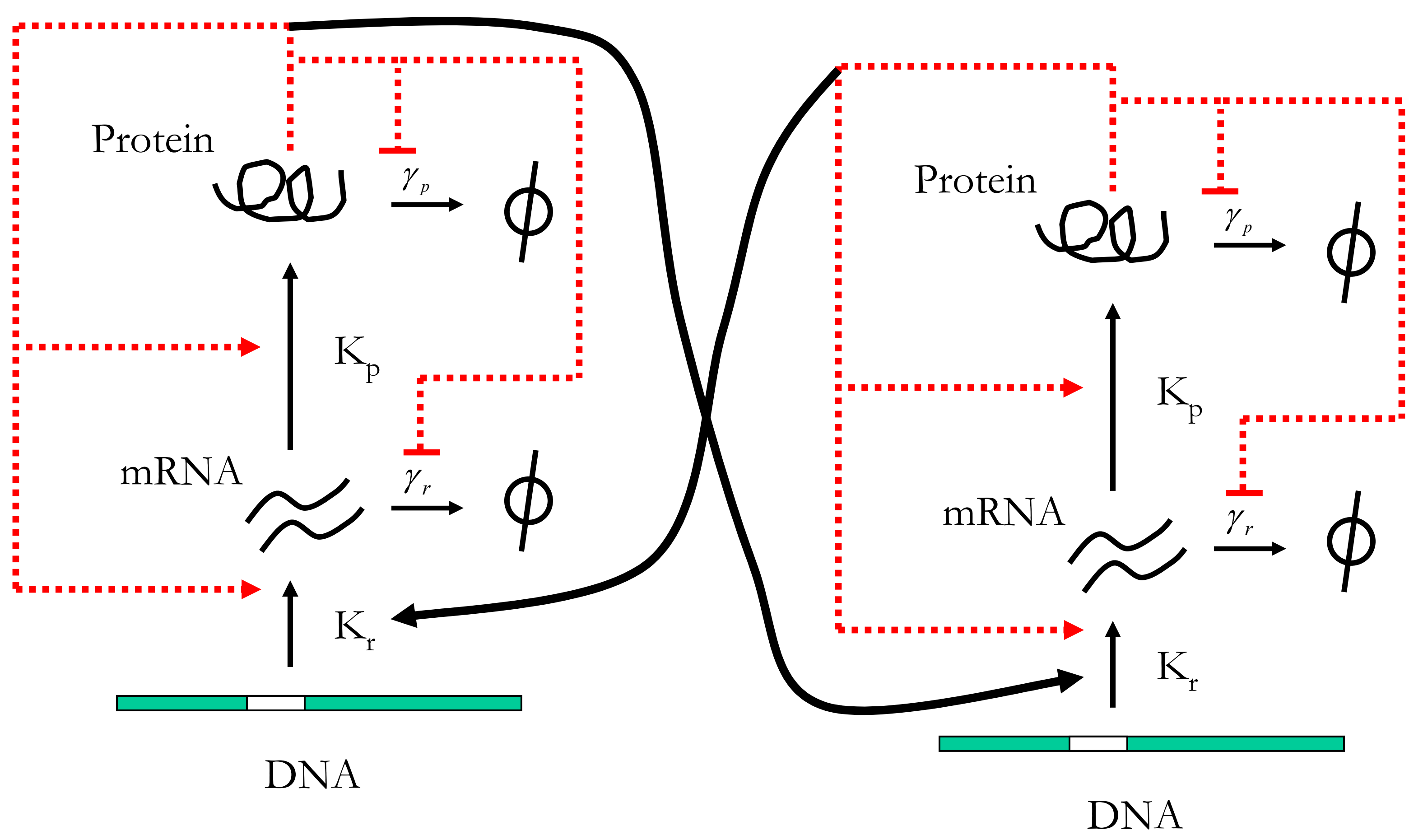
Rich scenarios are possible and should be investigated



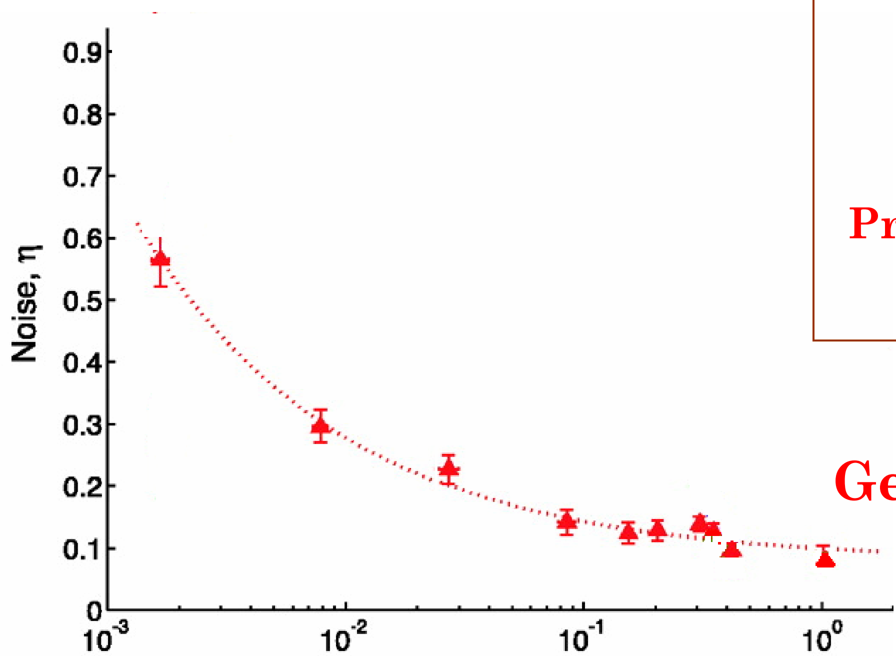
Example: Heat shock response



And of course, local nested loops of positive and negative feedback and global loops to connect different sub-systems



Number of Molecules



Relative Fluorescence Proportional to number Of molecules

M. Elowitz *et. al,* Stochastic Gene Expression in a Single Cell, *Science,* 2002.

``Models of stochastic Gene expression predict that intrinsic noise should increase as the amount of transcript decreases''

Proof: noise levels increase as square root of number of molecules

False Generalization

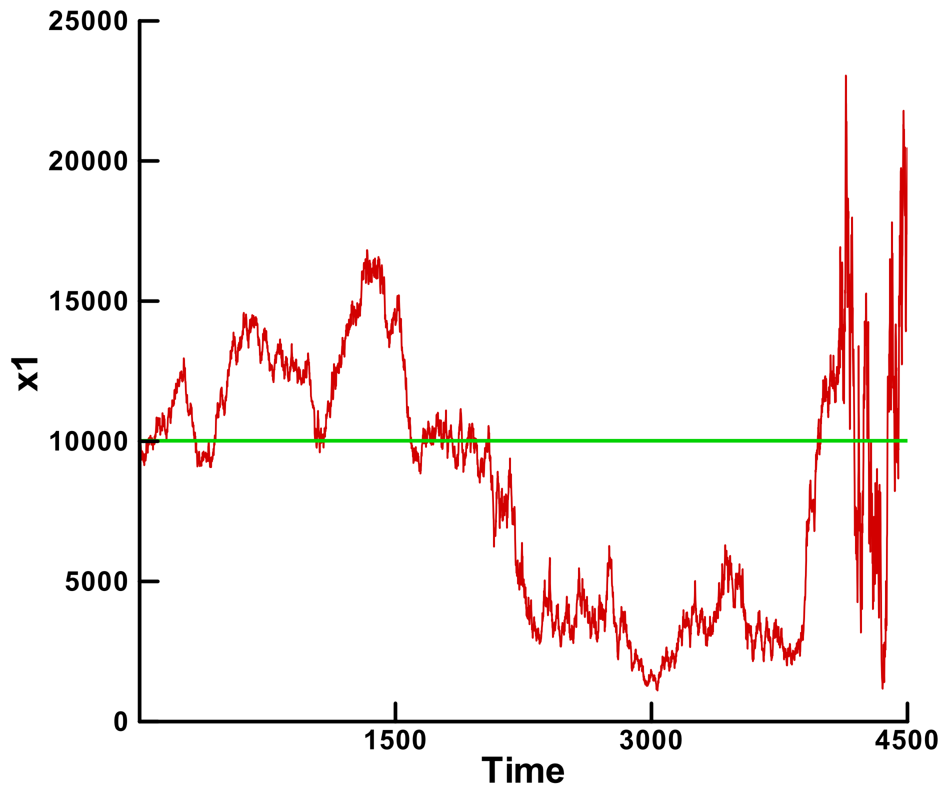
Large number of molecules is a sufficient condition for good noise attenuation in gene networks

Large Number of Molecules is Not Sufficient for Noise Attenuation

$$A \xrightarrow{k} x_1, B \xrightarrow{k} x_2$$

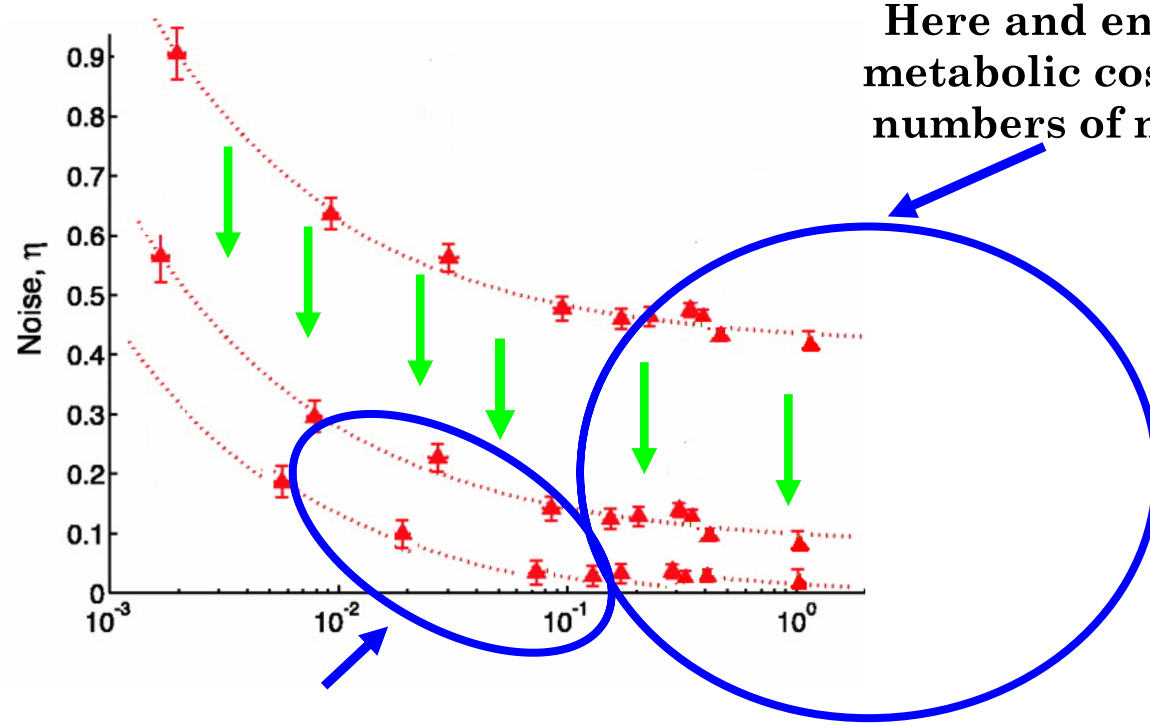
$$x_1 \xrightarrow{\alpha_1} \phi, x_2 \xrightarrow{\alpha_2} \phi$$

$$x_1 + x_2 \xrightarrow{k_a} C$$



Small Number of Molecules Does Not Necessarily Yield substantial Noise Amplification

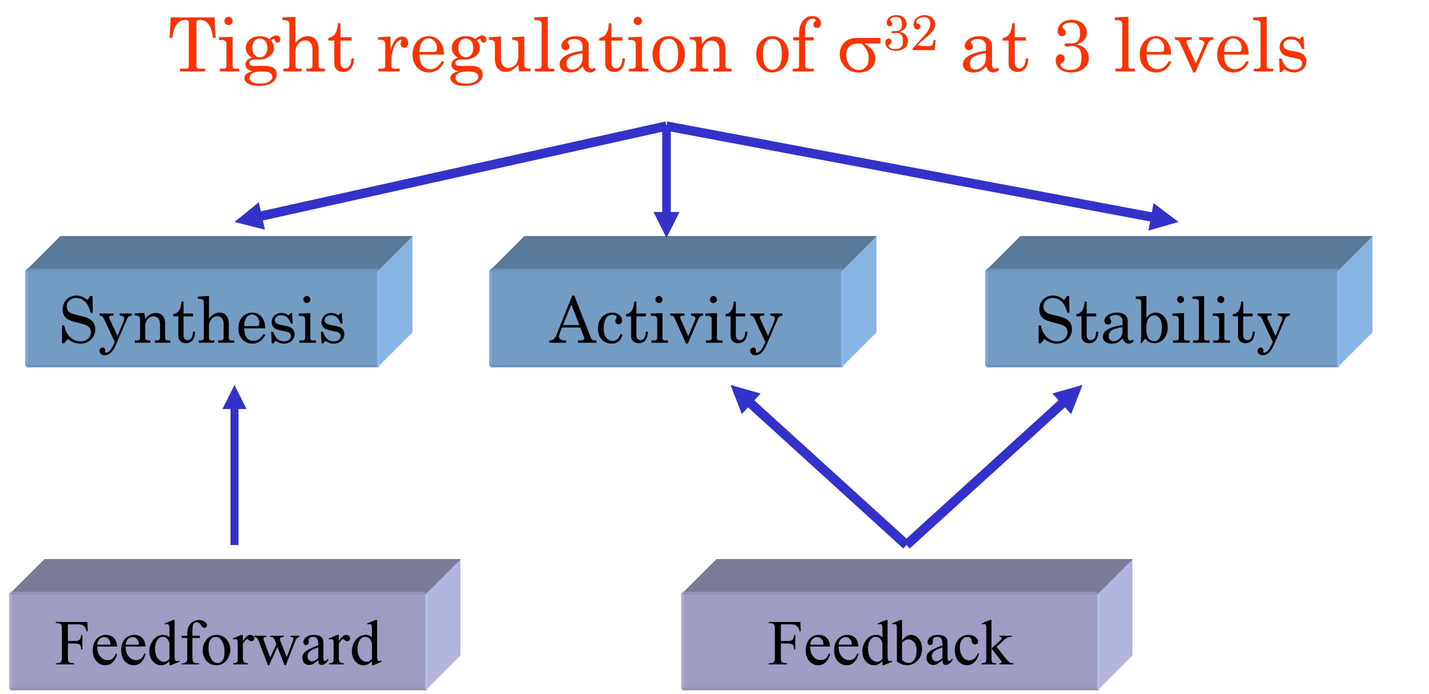
Gene networks can operate Here and endure the metabolic cost of large numbers of molecules

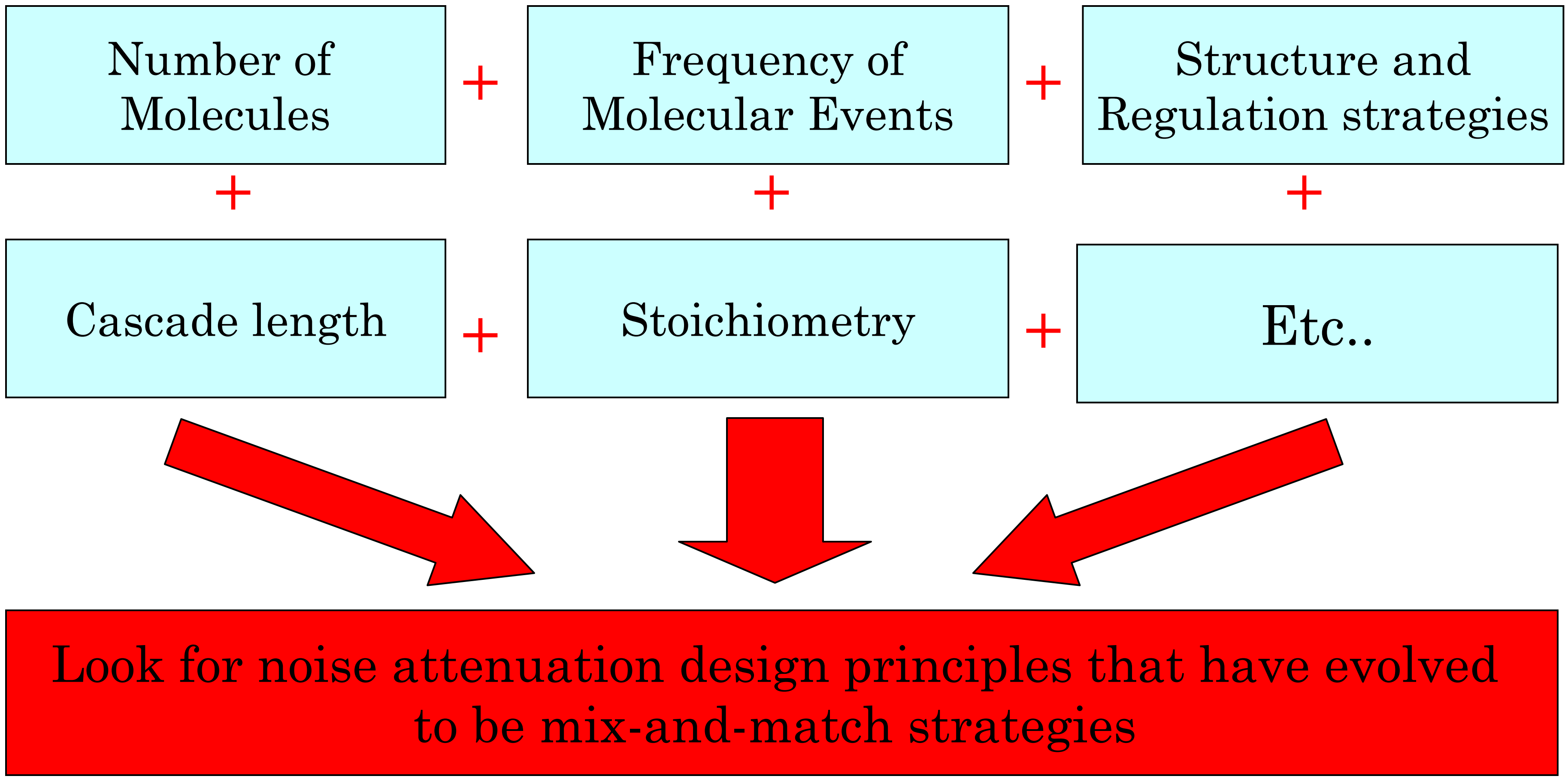


Or operate at small number of molecules
And push the noise level down here by using feedback and other design options

Example: Heat Shock Response

- **Number of sigma factor 30-50/cell**
- **Strategy: low number of molecules, but tight feedback regulation.**
- **Result: robust, noise free, and reliable response**




Number of Molecules
+
Frequency of Molecular Events
+
Structure and Regulation strategies
+
+
+
Cascade length
+
Stoichiometry
+
Etc..
Look for noise attenuation design principles that have evolved to be mix-and-match strategies

Conclusions

- Extraction and cataloguing of design principles is essential
- Otherwise: Complexity is overwhelming
- Today: SOME design principles
- Many more
    - Modularity
    - Noise Exploitation
    - Mode of control: Inducible versus repressive
    - Stochastic stability
    - Etc…
- This will be another story for another day

Heat Shock References

- **Surviving Heat Shock: Control Strategies for Robustness and Performance,** H. El Samad, H. Kurata, J.C. Doyle, C.A. Gross and M. Khammash*, under review for PNAS.*
- **Systems Biology: From Physiology to Gene Regulation,** M. Khammash and H. El Samad, *To appear in the Control Systems Mag., Aug. 2004*.
- **Stochasticity, Intrinsic Noise, and Regulation of Protein Stability in a Model of the Heat Shock Response,** H. El Samad and M.Khammash, *available as preprint*
- **Feedback control of the heat shock response in E. coli**, H. Kurata, H. El-Samad, T.-M. Yi, M. Khammash, J. C. Doyle, *Proceedings of the Conference on Decision and Control, 2001*.
- **Sensitivity analysis of the heat shock response in E. coli,** H. El-Samad, M. Khammash, H. Kurata, J. C. Doyle*, Proceedings of the American Control Conference, 2002.*
- **Model Validation and Robustness Analysis of the Bacterial Heat Shock Response Using SOSTOOLS,** H. El Samad, S.Prajna, A. Papachristodolou, M. Khammash, J.C. Doyle, *Proceedings of Conference on Decision and Control, 2003*.

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Premise: **``A Promoter that undergoes frequent activation steps followed by inefficient transcription will produce a cellular population with little variability, whereas a promoter that undergoes infrequent activation followed by efficient transcription can display large differences from cell to cell "**

Constrained Design Principles