Ultrasensitivity and cellular decisions

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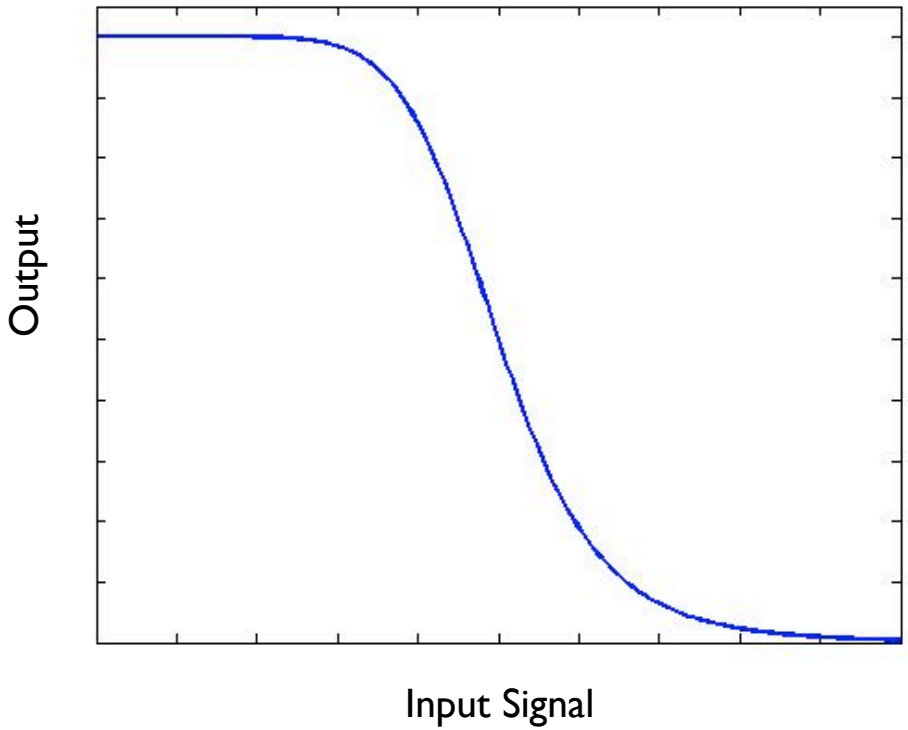
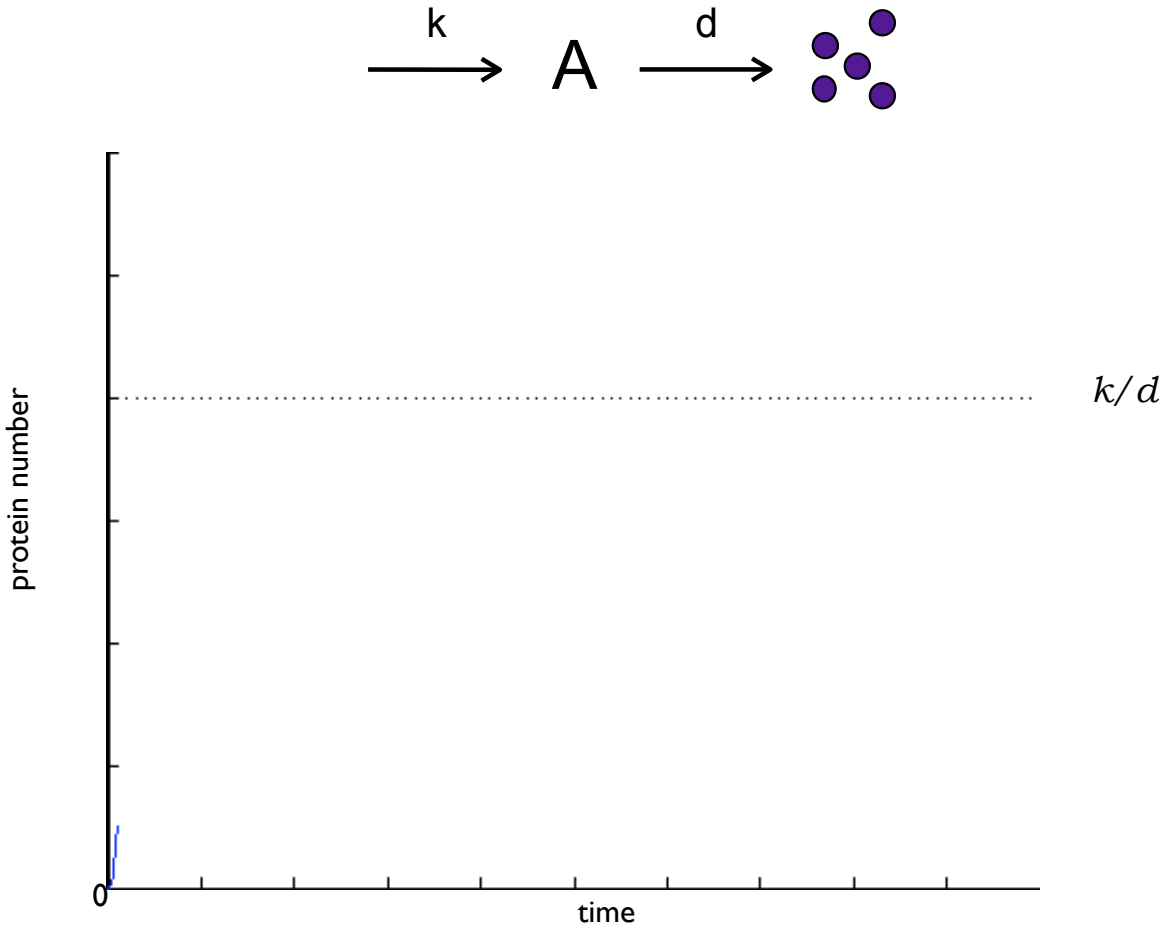
Many biochemical networks have sigmoidal, or ultrasensitive, responses.
Why?



Cells must make decisions from sensing stochastic signals using stochastic biochemistry because all chemistry is stochastic.

For example, consider a rudimentary model of gene expression:



Substantial stochasticity has been measured in the biochemistry of many organisms:

Humans

Variability and memory of protein levels in human cells

Alex Sigal[1]*, Ron Milo[1]*†, Ariel Cohen[1]*, Naama Geva-Zatorsky[1], Yael Klein[1], Yuvalal Liron[1], Nitzan Rosenfeld[1], Tamar Danon[1], Natalie Perzov[1] & Uri Alon[1]

Yeast

Control of Stochasticity in Eukaryotic Gene Expression

Jonathan M. Raser and Erin K. O'Shea*

Slime moulds

Transcriptional Pulsing of a Developmental Gene

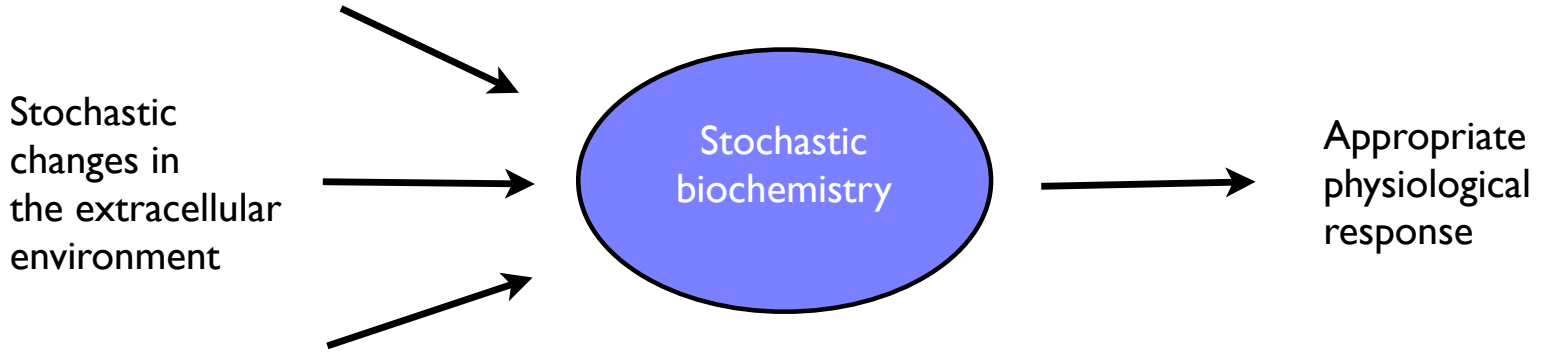
Jonathan R. Chubb,[1,2,*] Tatjana Trcek,[1] Shailesh M. Shenoy,[1] and Robert H. Singer[1]

Bacteria

Stochastic Gene Expression in a Single Cell

Michael B. Elowitz,[1,2*] Arnold J. Levine,[1] Eric D. Siggia,[2] Peter S. Swain[2]

What strategies do cells have for their decision-making?



By strategy, I mean how a signalling network detects and analyses information not in terms of the details of biochemistry but in terms of the functions of information-processing that biochemistry performs.

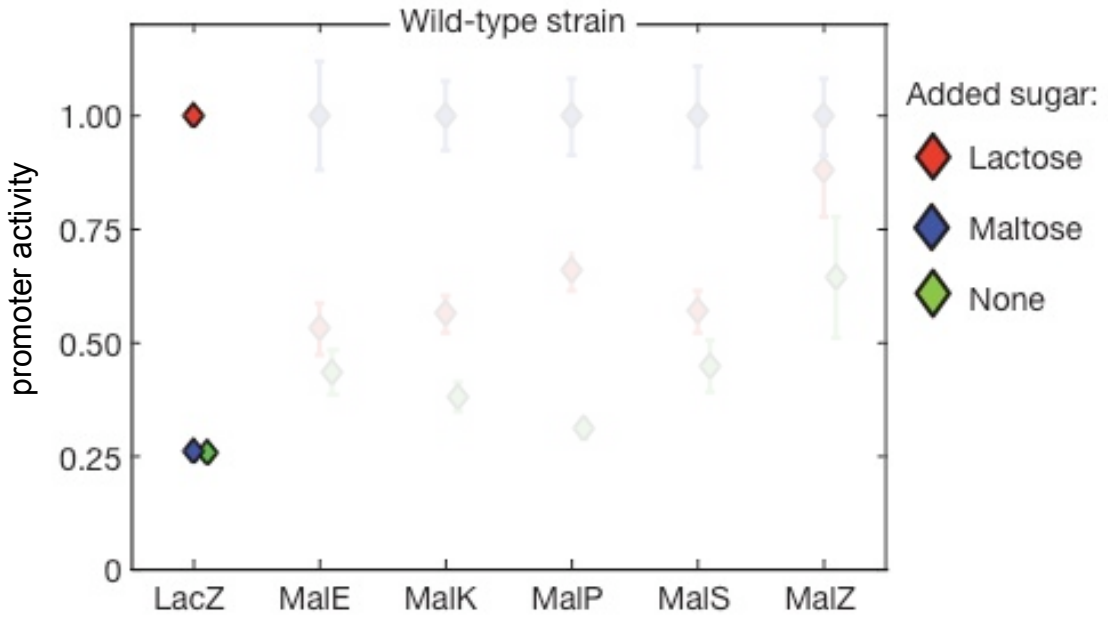
What are cells learning from sensing?

Single cells can use signals to anticipate a change in the state of their environment.

In our intestine, bacteria are exposed to the sugar lactose before the sugar maltose.

Adaptive prediction of environmental changes by microorganisms

Amir Mitchell[1], Gal H. Romano[2], Bella Groisman[1], Avihu Yona[1], Erez Dekel[3], Martin Kupiec[2], Orna Dahan[1]* & Yitzhak Pilpel[1,4]*



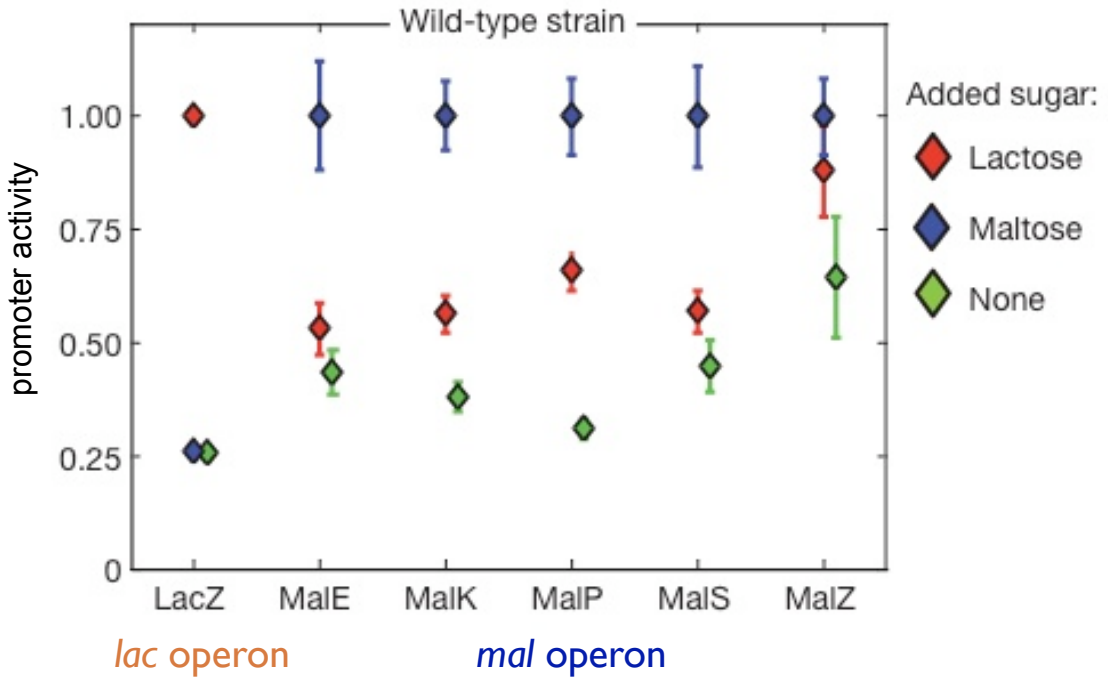
lac operon

Single cells can use signals to anticipate a change in the state of their environment.

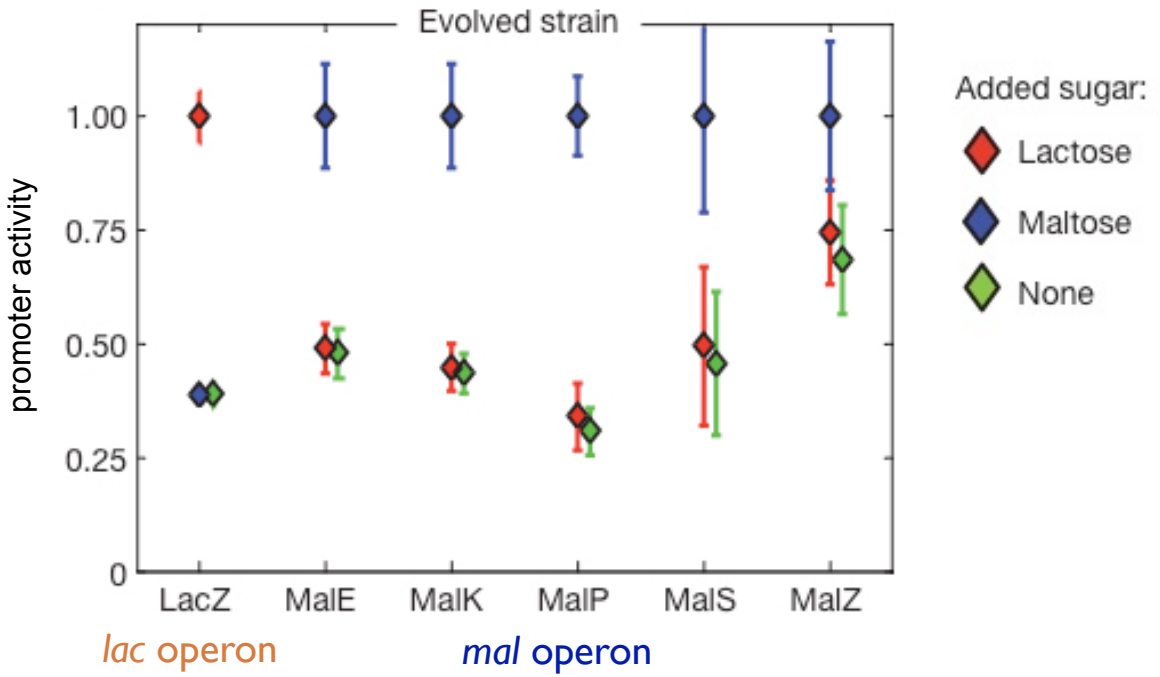
In our intestine, bacteria are exposed to the sugar lactose before the sugar maltose, but use the presence of lactose to predict the imminent occurrence of maltose.

Adaptive prediction of environmental changes by microorganisms

Amir Mitchell[1], Gal H. Romano[2], Bella Groisman[1], Avihu Yona[1], Erez Dekel[3], Martin Kupiec[2], Orna Dahan[1]* & Yitzhak Pilpel[1,4]*



The predictive response is adaptive: bacteria grown in environments where lactose is not followed by maltose no longer predict the occurrence of maltose upon exposure to lactose.

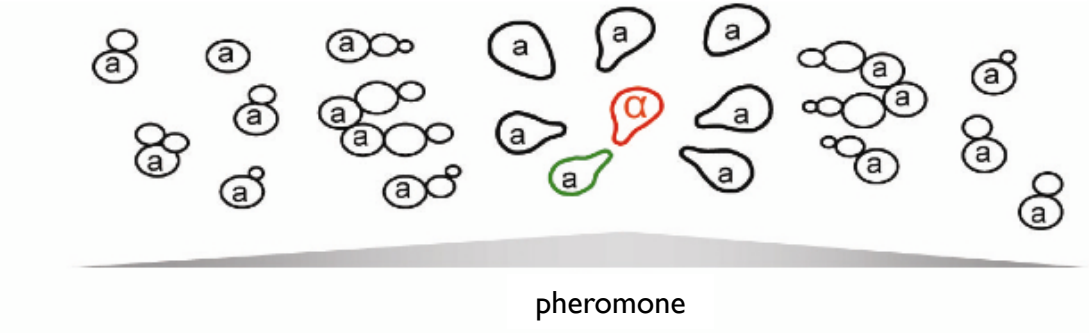


How might cells infer the state of the extracellular environment?

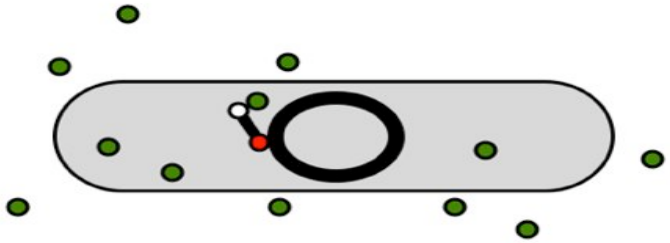
Can we understand the “design” of the biochemistry that allows them to do so?

We will consider two apparently different, yet similar, examples:

The mating response in budding yeast

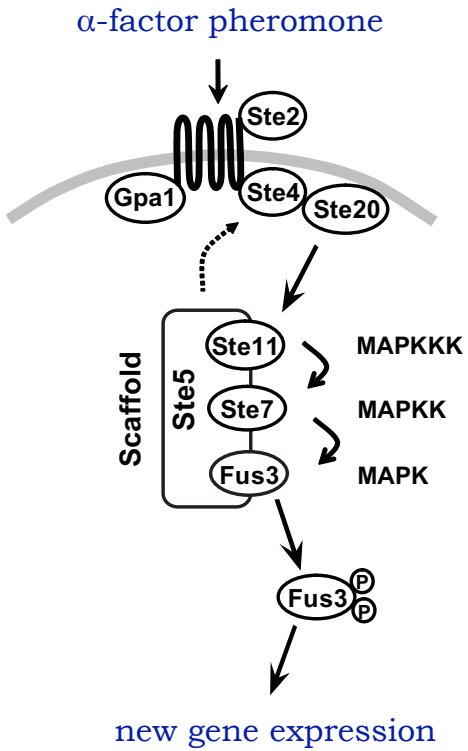


Gene expression in bacteria

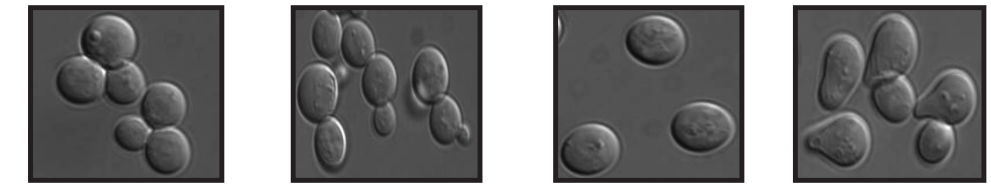


Control of the mating response of budding yeast

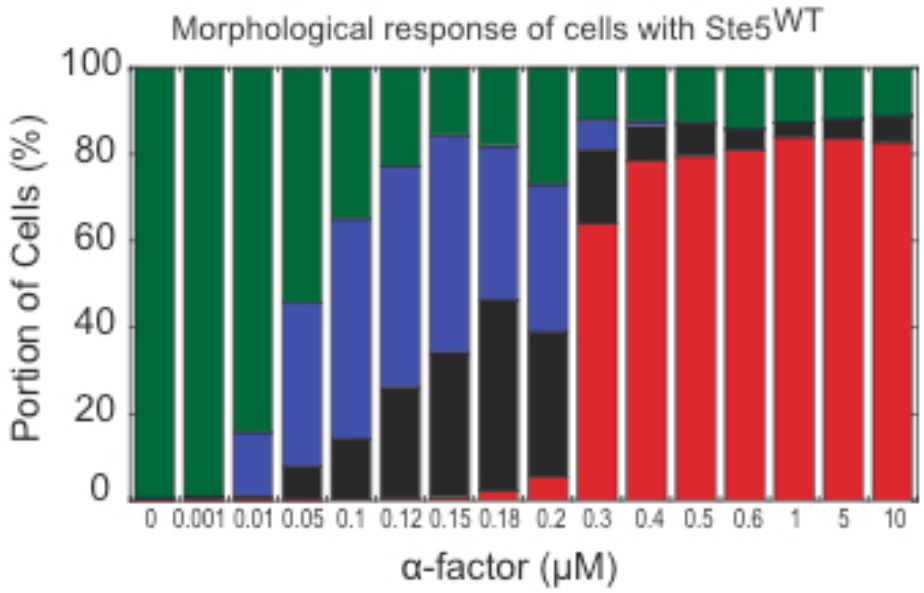
Signal transduction: the mating response in budding yeast is controlled by a scaffolded cascade of MAP kinases.



The fraction of shmooing cells varies sigmoidally with the concentration of pheromone.

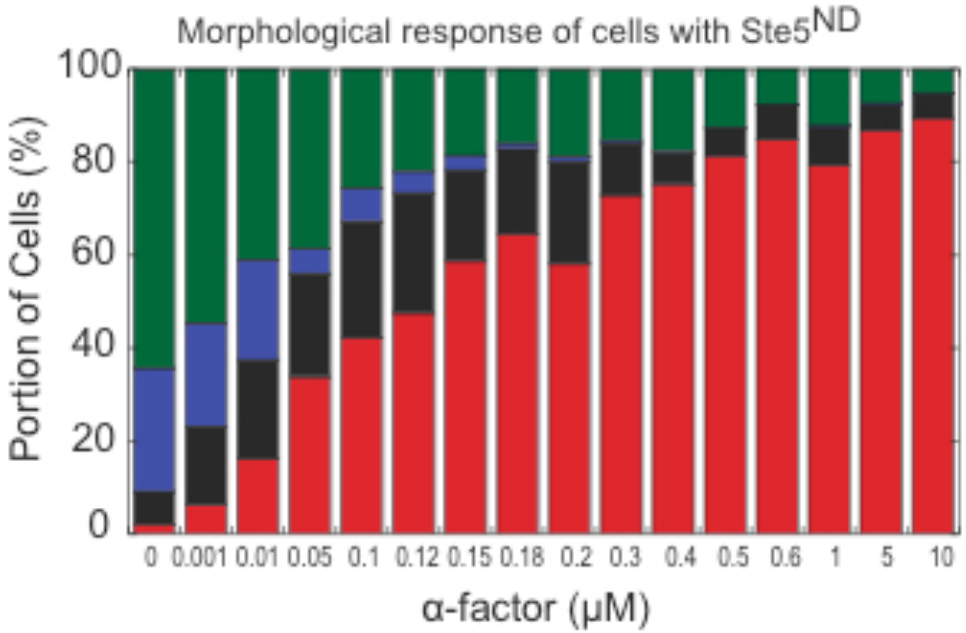


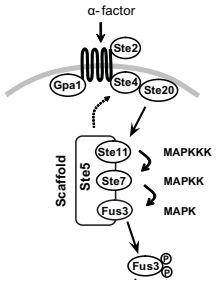
Axial budding | Bipolar budding | Cell cycle arrested | Shmooing



n_H=9

The scaffold Ste5 plays a role in generating the ultrasensitivity.

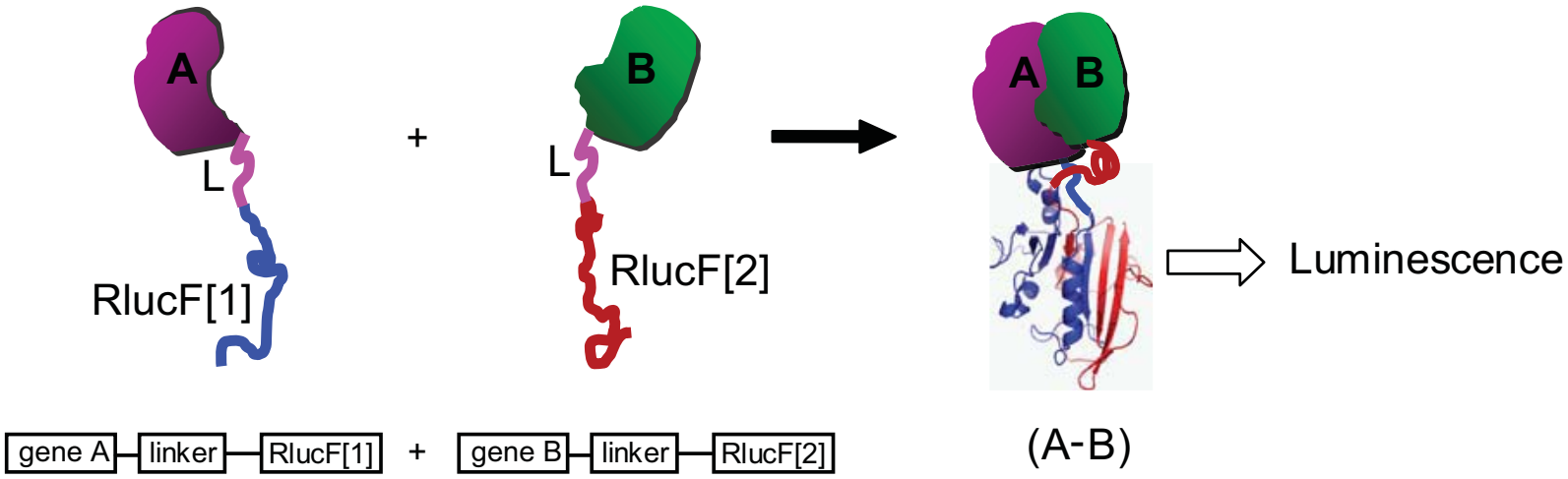




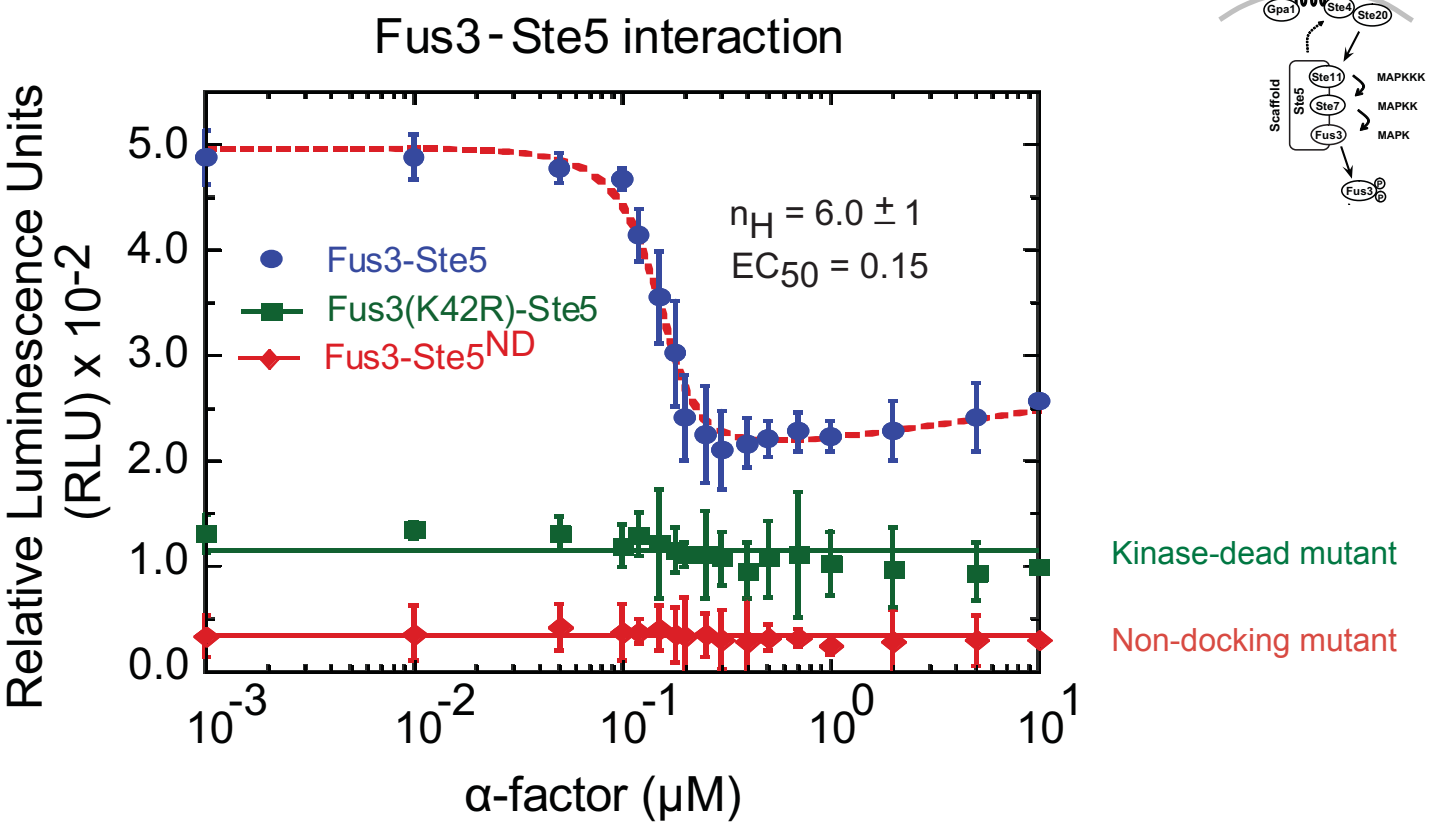
n_H=1.3

The mutant Ste5ND does not bind the kinase Fus3.

We use a protein-fragment complementation assay (PCA) based on *Renilla* luciferase to measure interactions between proteins *in vivo*.

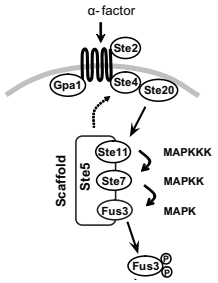


A PCA assay of the interaction between the MAP kinase Fus3 and the scaffold Ste5 shows that the kinase activity of Fus3 is necessary to generate the ultrasensitivity.



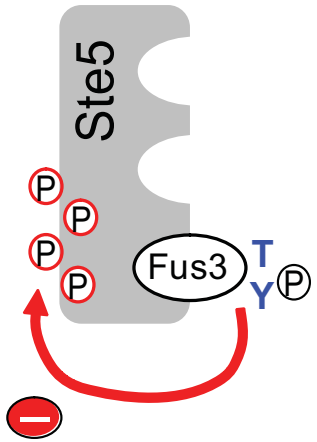
The scaffold Ste5 activates auto-phosphorylation of the MAP kinase Fus3, but such partially active Fus3 inhibits mating by promoting phosphorylation of Ste5 and so increases the apparent binding affinity between Fus3 and Ste5.

Bhattacharyya *et al.*, Science 2006



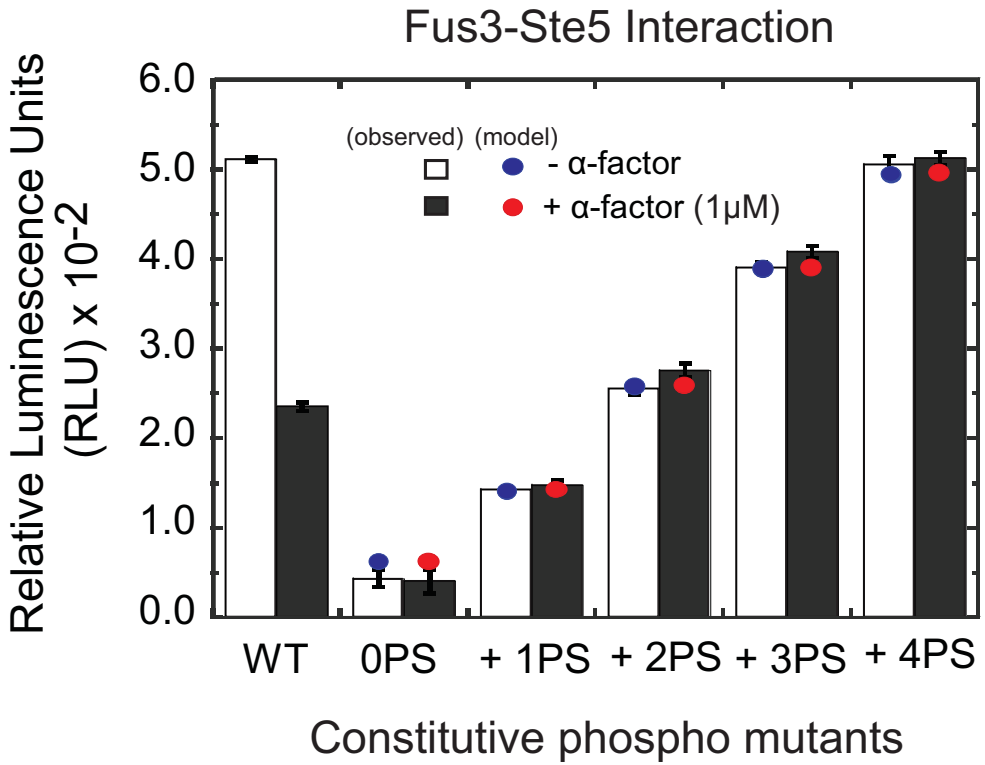
Negative recruitment

-α-factor

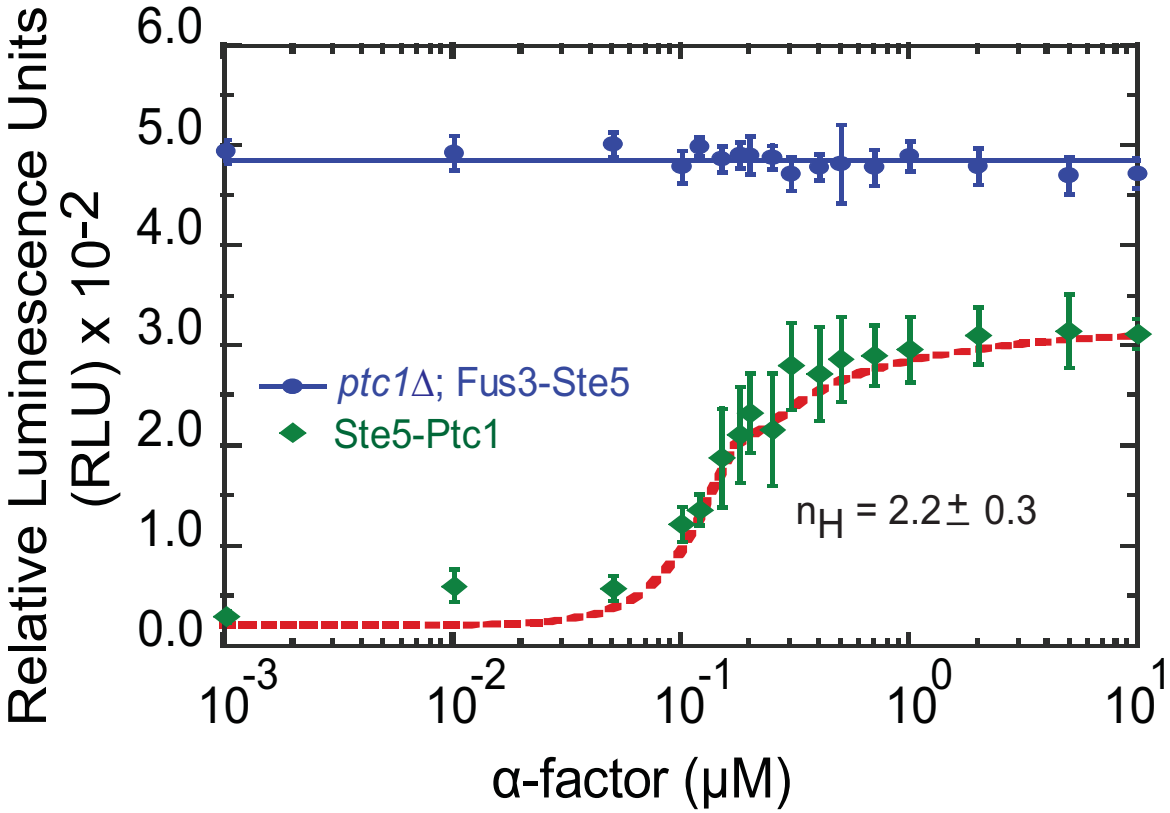


We find 3 new potential sites on Ste5 for phosphorylation by Fus3.

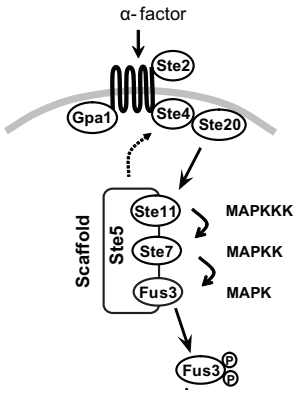
Adding back constitutively phosphorylated sites increases the interaction between Ste5 and Fus3, but it is no longer regulated by α-factor.



How does the MAP kinase Fus3 dissociate from the scaffold Ste5? The phosphatase Ptc1 is recruited to Ste5 as the concentration of α-factor increases.



How is the sigmoidal response in the interaction between the scaffold Ste5 and the MAP kinase Fus3 generated?



In the absence of pheromone, Fus3 binds to Ste5, becomes partially active, phosphorylates Ste5, and then binds strongly to phosphorylated Ste5, but a phosphatase Ptc1 is recruited to Ste5 as pheromone increases.

Proc. Natl. Acad. Sci. USA
Vol. 78, No. 11, pp. 6840–6844, November 1981
Biochemistry

An amplified sensitivity arising from covalent modification in biological systems

(protein modification/metabolic regulation/switch mechanism/enzyme cascades)

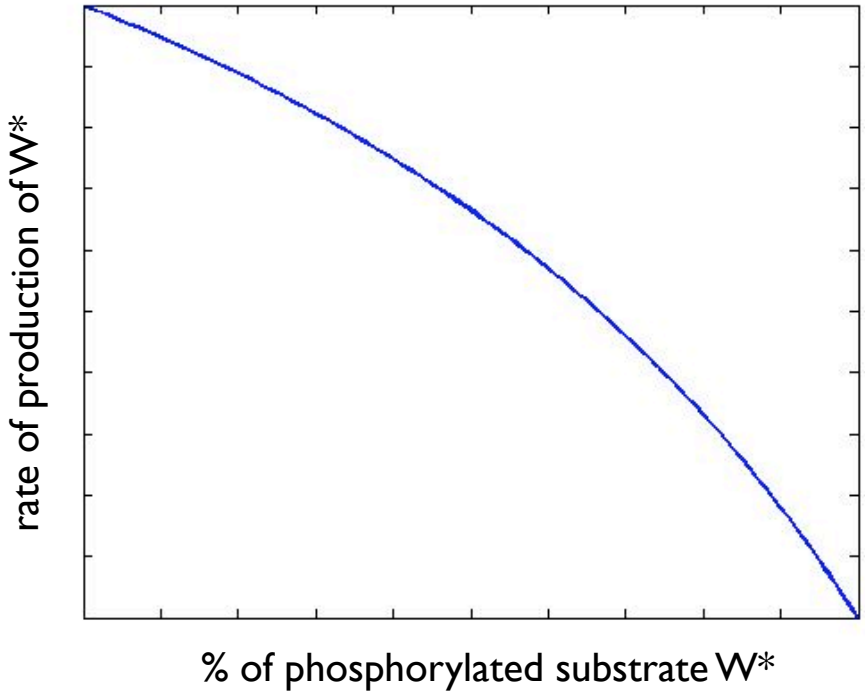
ALBERT GOLDBETER† AND DANIEL E. KOSHLAND, JR.

Zero-order ultrasensitivity from a competition between two opposing enzymes: a kinase and a phosphatase

ABSTRACT The transient and steady-state behavior of a reversible covalent modification system is examined. When the modifying enzymes operate outside the region of first-order kinetics, small percentage changes in the concentration of the effector controlling either of the modifying enzymes can give much larger percentage changes in the amount of modified protein. This amplification of the response to a stimulus can provide additional sensitivity in biological control, equivalent to that of allosteric proteins with high Hill coefficients.

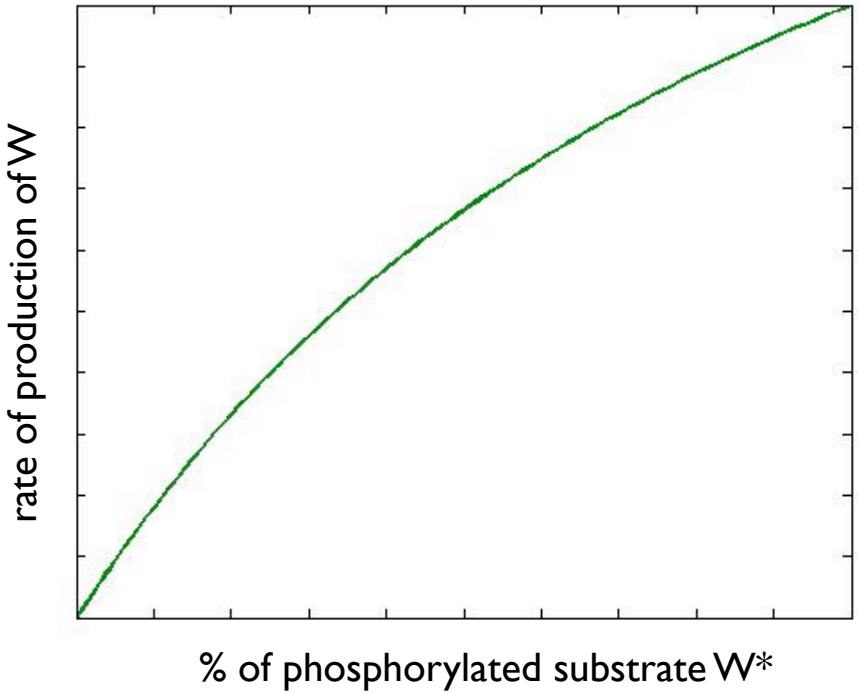
Consider a kinase acting on a substrate:

$$W \longrightarrow W^*$$



Consider the action of a phosphatase if the kinase is de-activated:

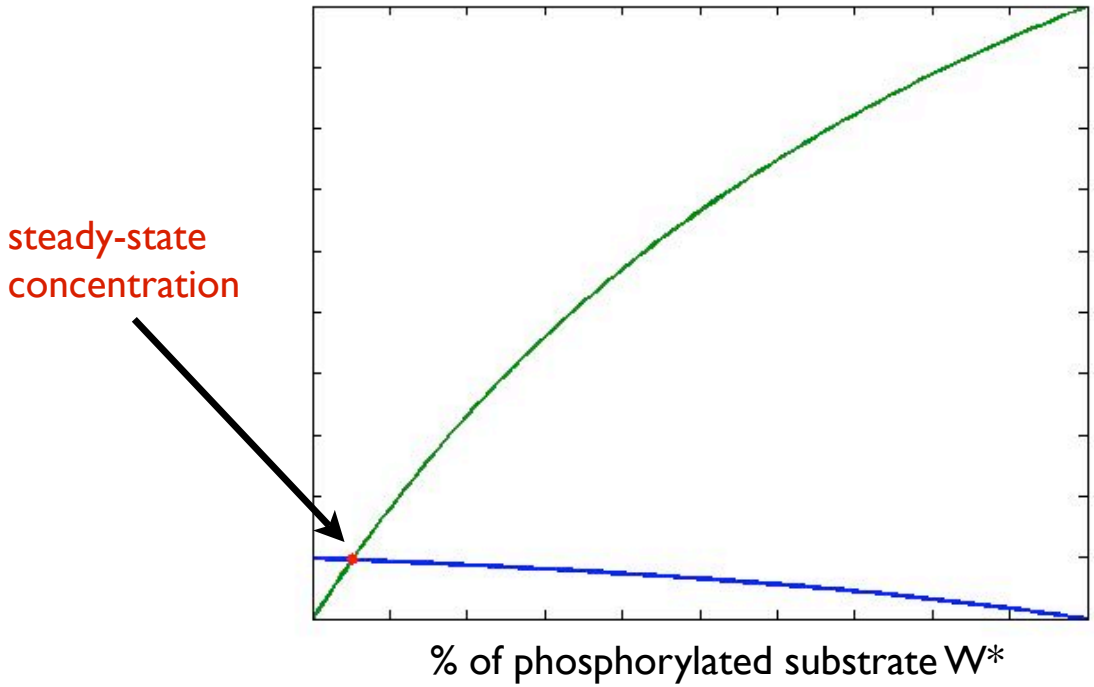
$$W^* \longrightarrow W$$



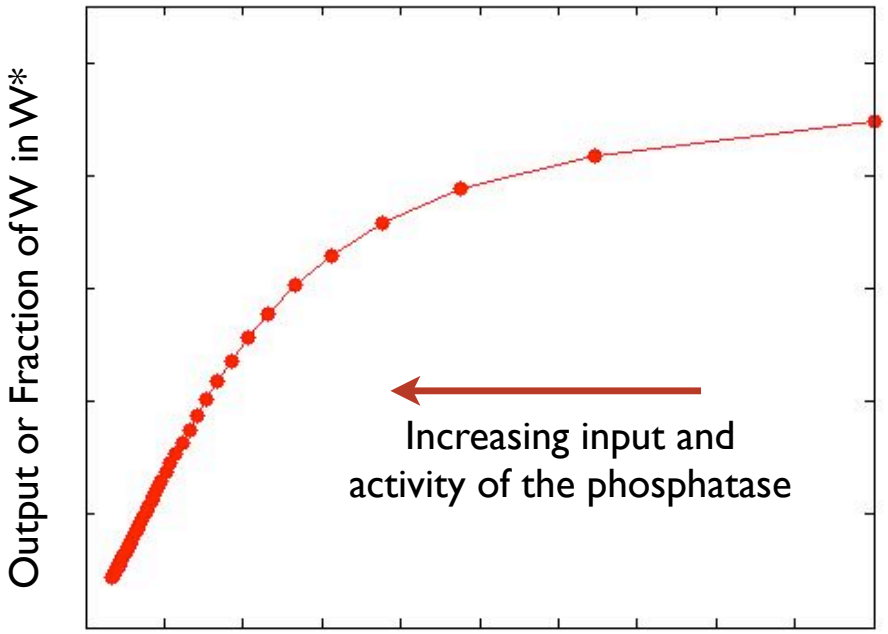
If both the kinase and the phosphatase act, the system reaches steady-state when the rate of production of W* by the kinase matches the rate of production of W by the phosphatase.

$$W \longrightarrow W^* \qquad \text{kinase}$$

$$W^* \longrightarrow W \qquad \text{phosphatase}$$



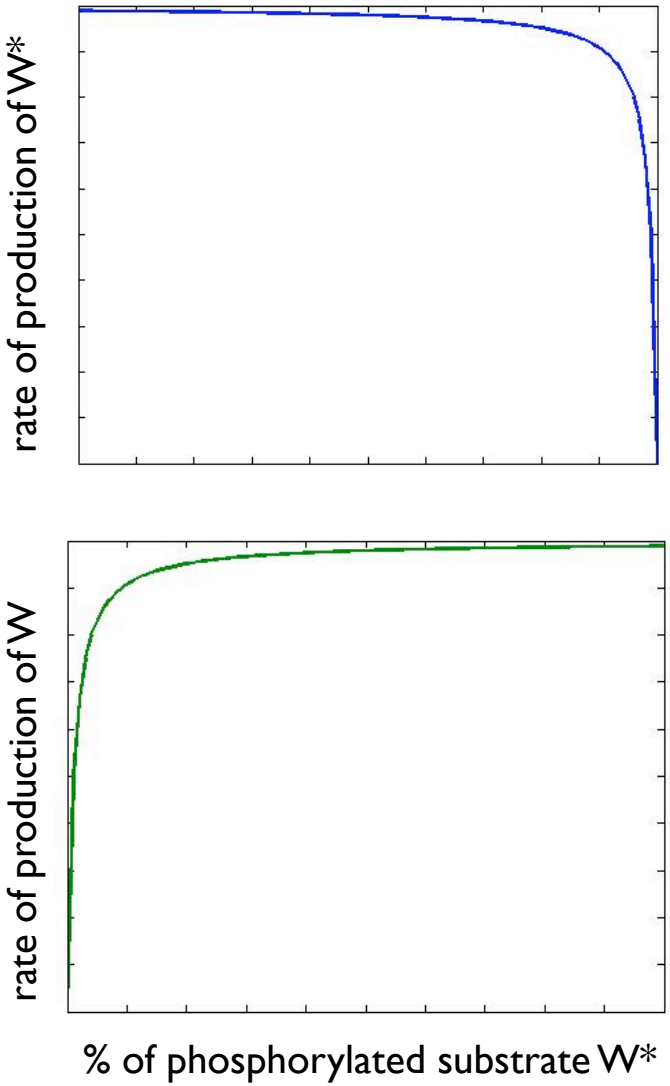
Follow the change in the steady-state concentration of W* as the activity of the phosphatase increases:



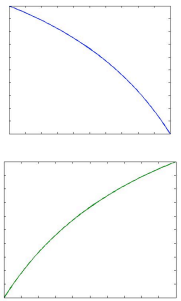
Hyperbolic response

$n_H = 1$

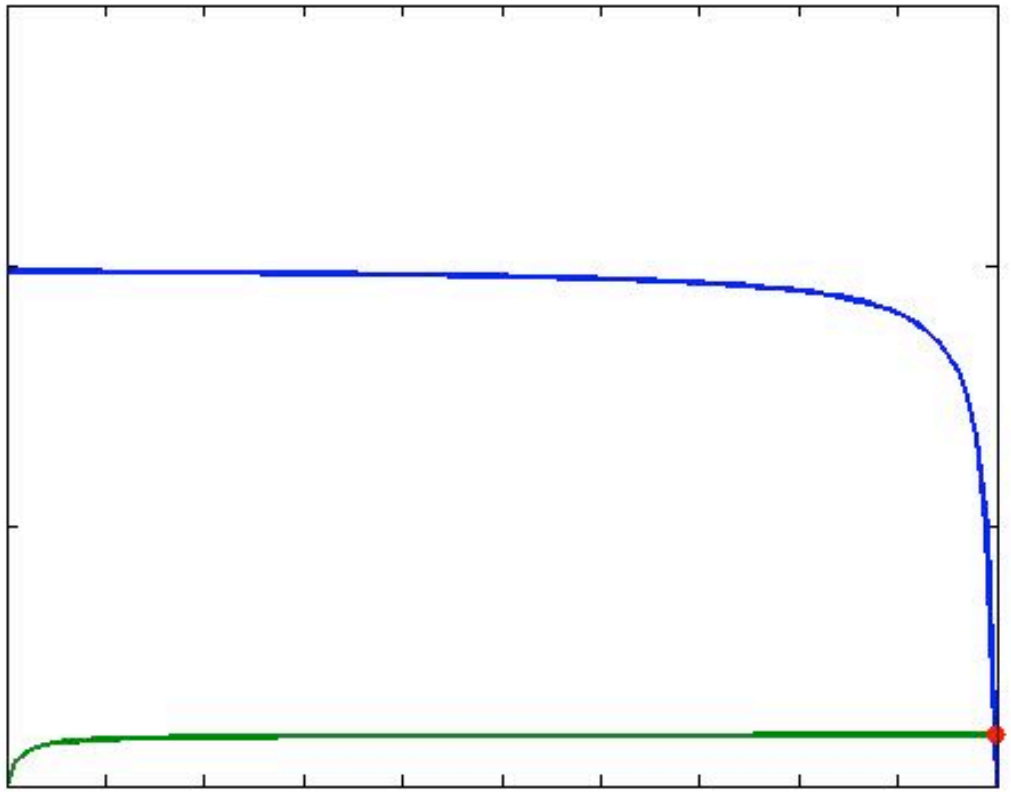
Let the amount of substrate potentially saturate the kinase and the phosphatase.



non-saturated enzymes

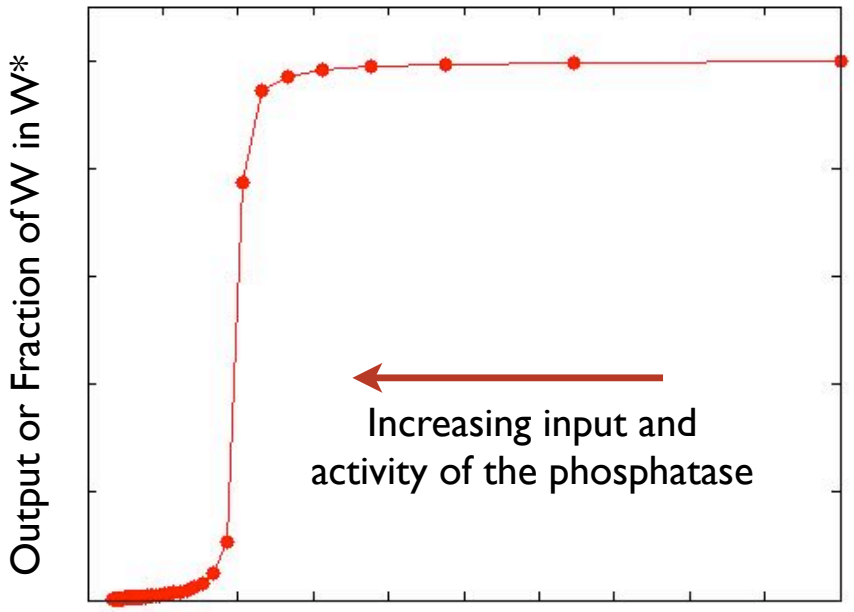


As we increase the concentration of the phosphatase, the steady-state concentration of W* changes sigmoidally.



% of phosphorylated substrate W*

The change in steady-state concentration of W* varies sigmoidally or ultrasensitively as the activity of the phosphatase increases.



When the enzymes work near saturation, the kinase is unable to compensate for increases in the activity of the phosphatase generating large changes in the steady-state concentration of W*.

Zero-order ultrasensitivity requires a kinase and a phosphatase competing for the same substrate, with both working at saturation.

But,

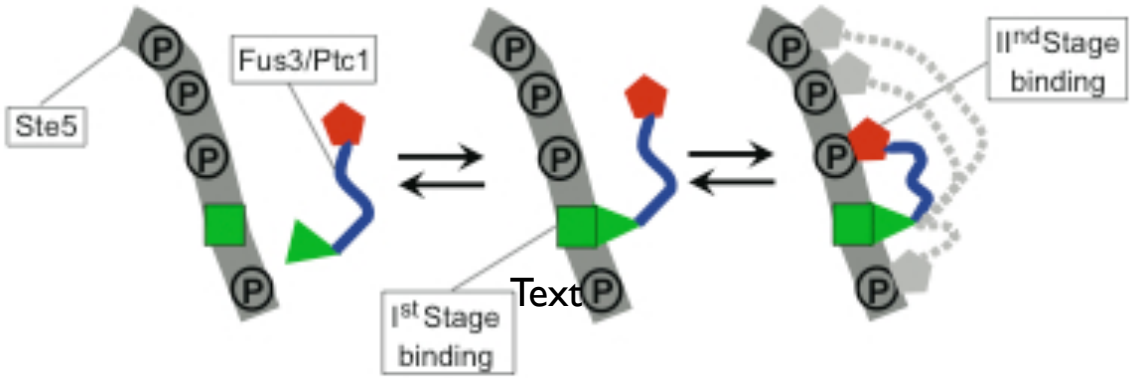
$$[\mathrm{Fus3}] \gg [\mathrm{Ste5}]$$

with

$$[\mathrm{Fus3}] \simeq 200\,\mathrm{nM}$$
$$[\mathrm{Ste5}] \simeq 50\,\mathrm{nM}$$

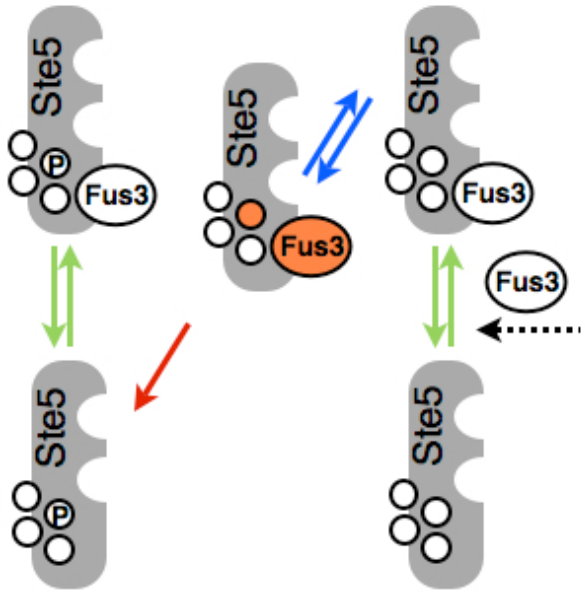
in vivo.

The kinase and phosphatase bind in two stages to the substrate Ste5.



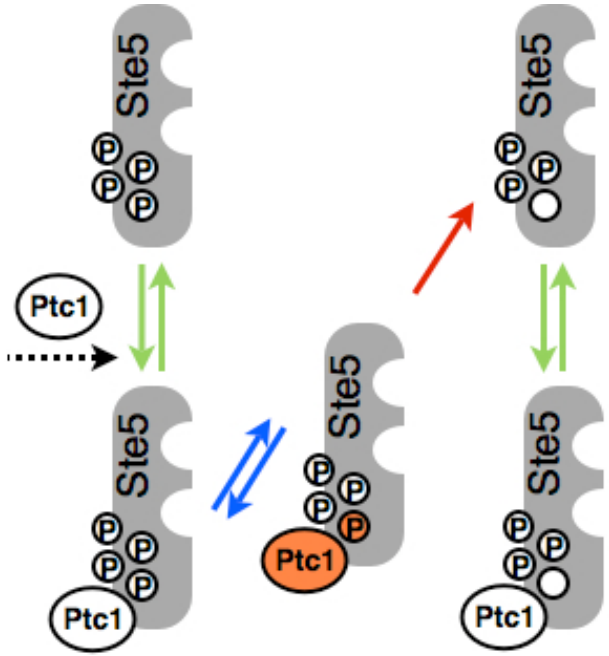
Each enzyme first binds to a docking motif on Ste5 before binding and then potentially performing an enzymatic reaction on a phosphosite.

Two-stage binding and multiple phosphosites on Ste5 implies local saturation of an individual Fus3 when bound to Ste5.

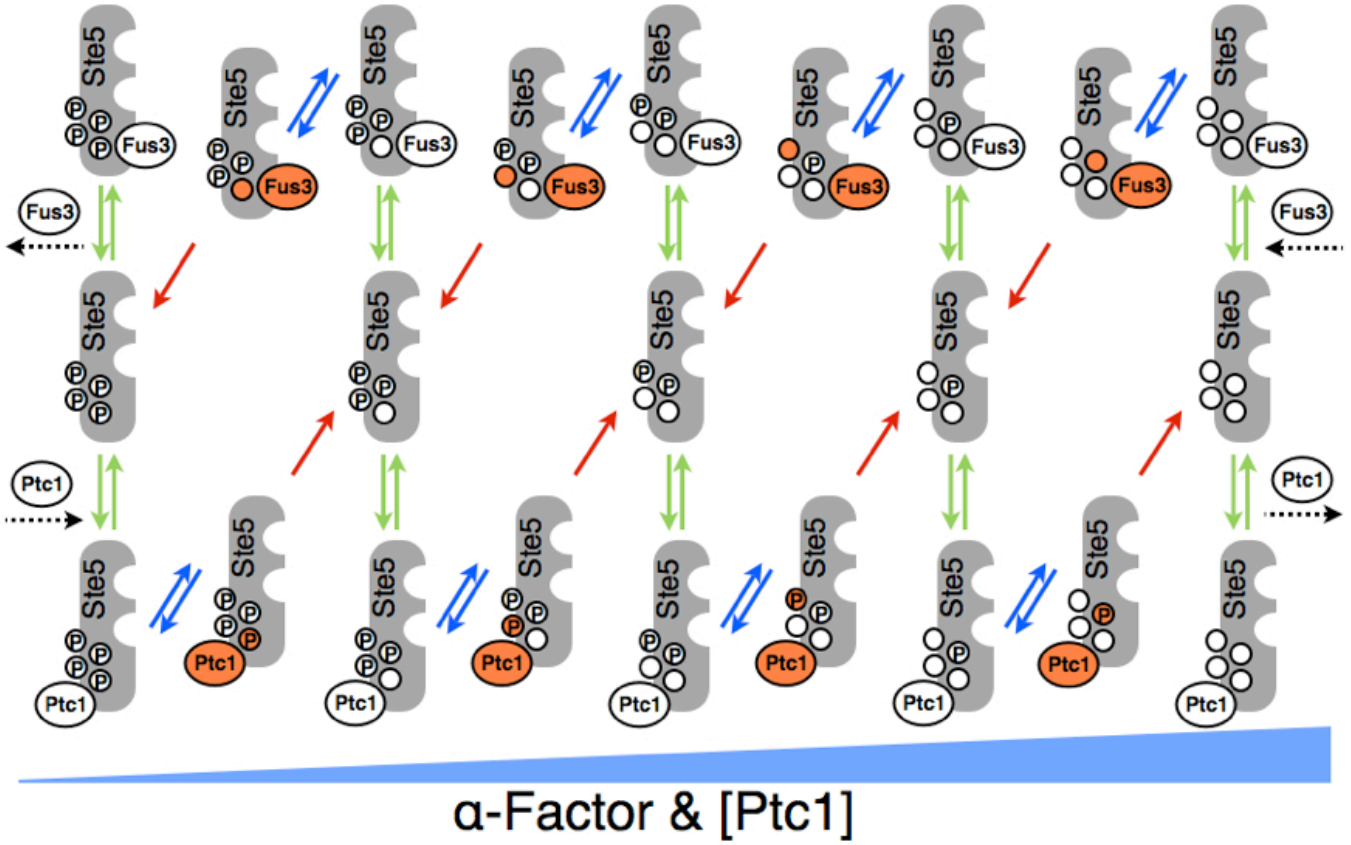


An enzyme is locally saturated on Ste5 when the probability of an enzyme binding to a phosphosite rather than dissociating from Ste5 is close to one.

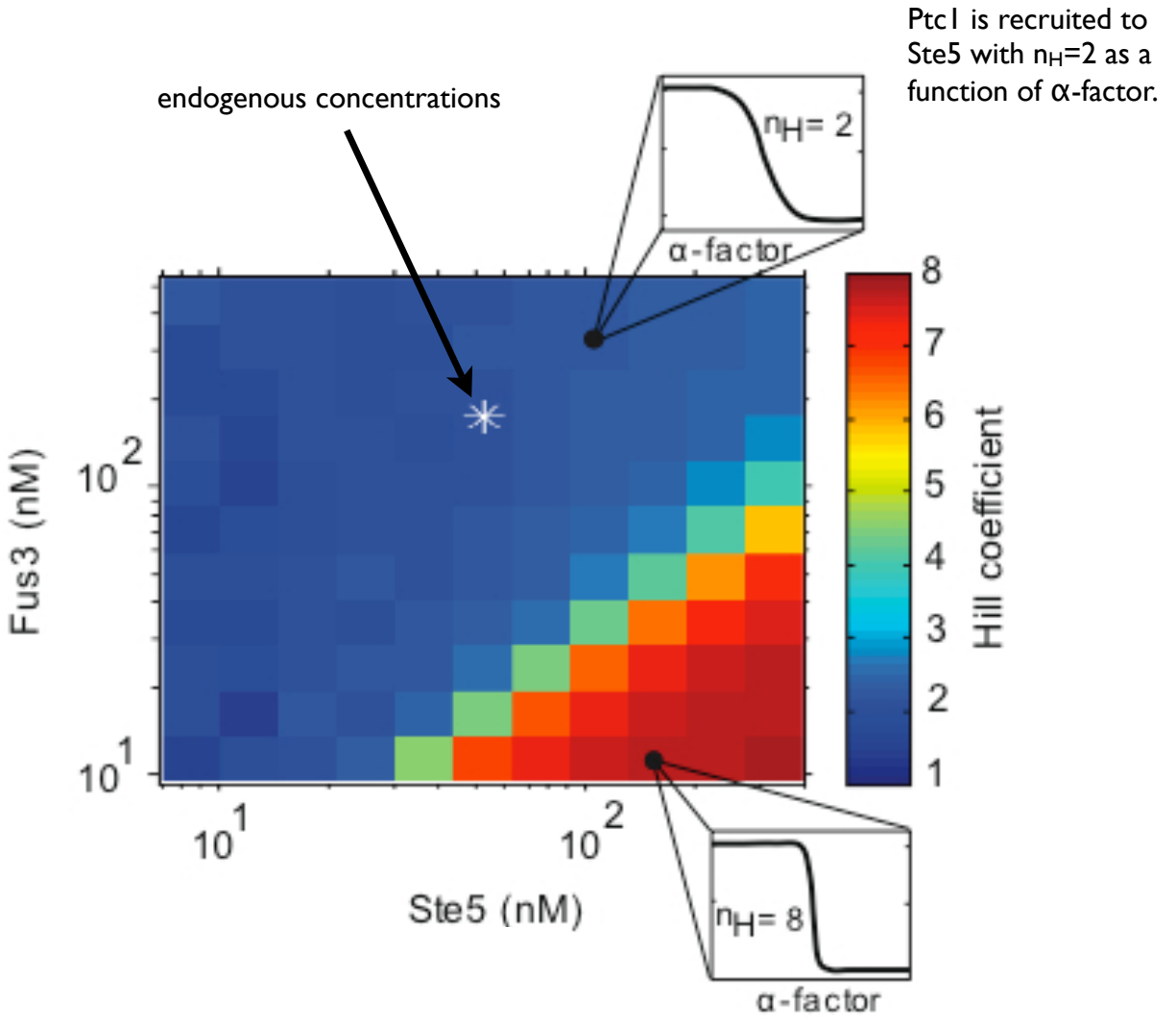
We assume that Ptc1 is similarly locally saturated through two-stage binding.



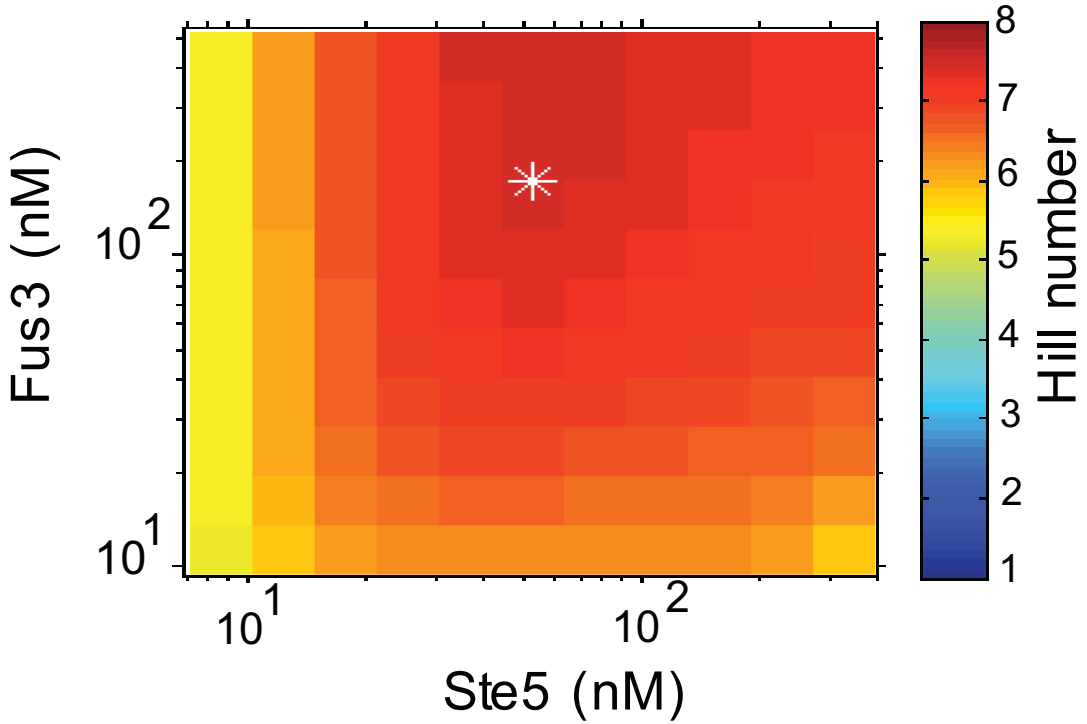
The full model with all 4 phosphosites on Ste5 and with Fus3 and Ptc1



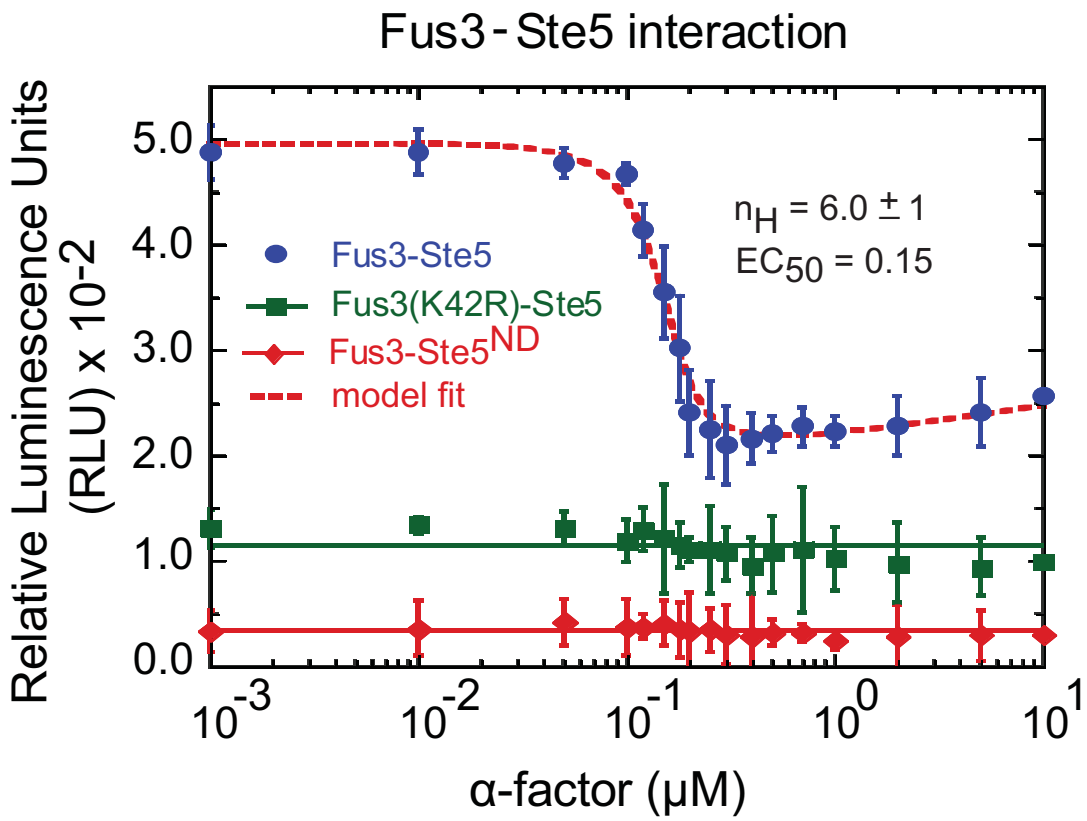
With only one phosphosite on Ste5, ultrasensitivity is not robust to changes in the concentrations of Ste5 and Fus3.



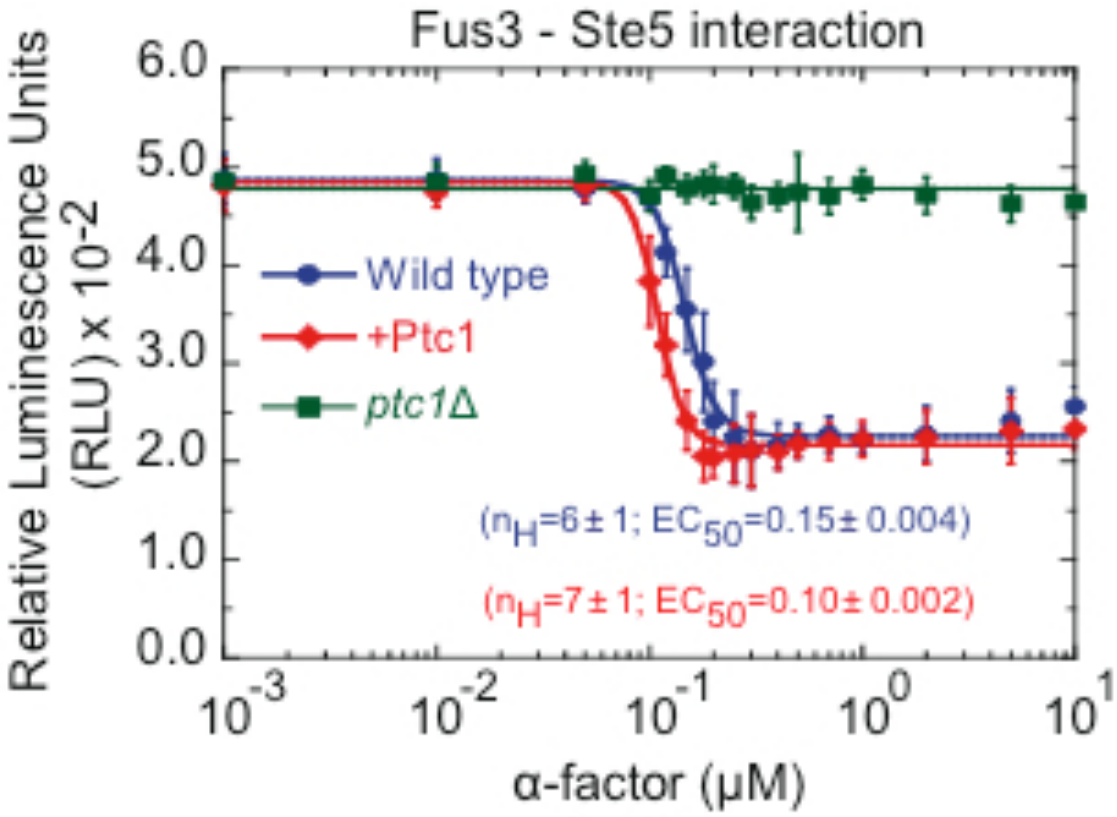
However, zero-order ultrasensitivity generated by two-stage binding and four phosphosites on Ste5 gives robust ultrasensitivity.



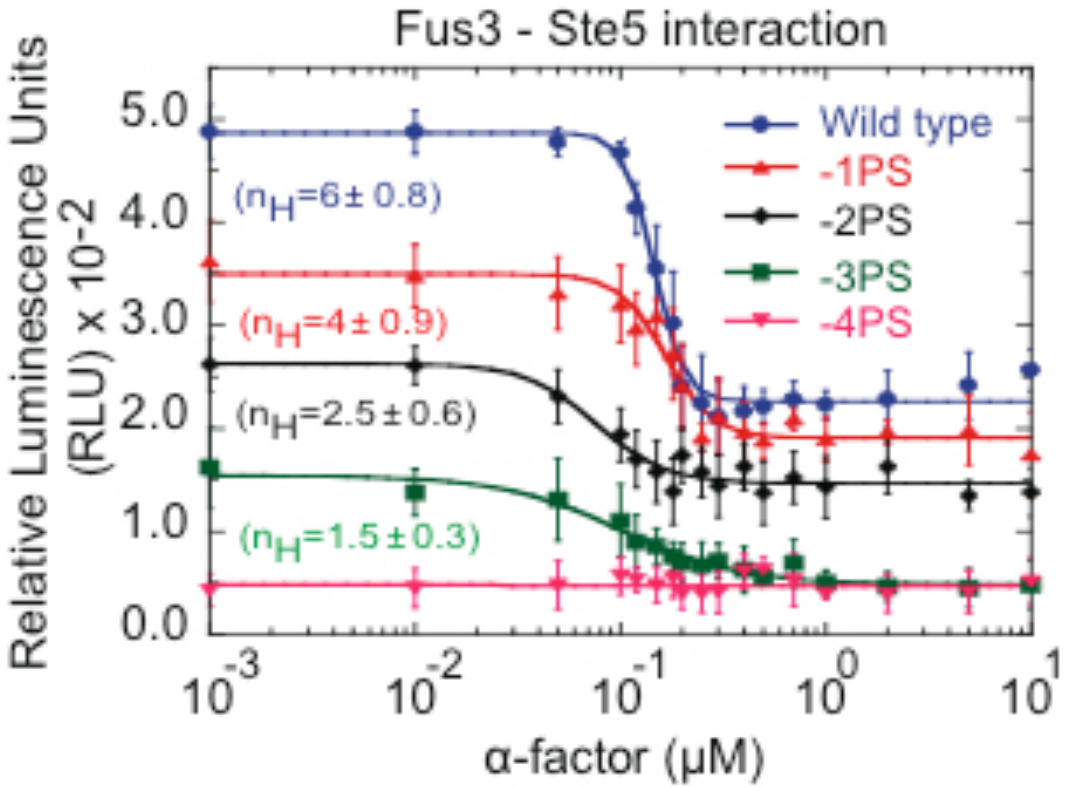
We can then reproduce the sigmoidal response detected by PCA with our model.



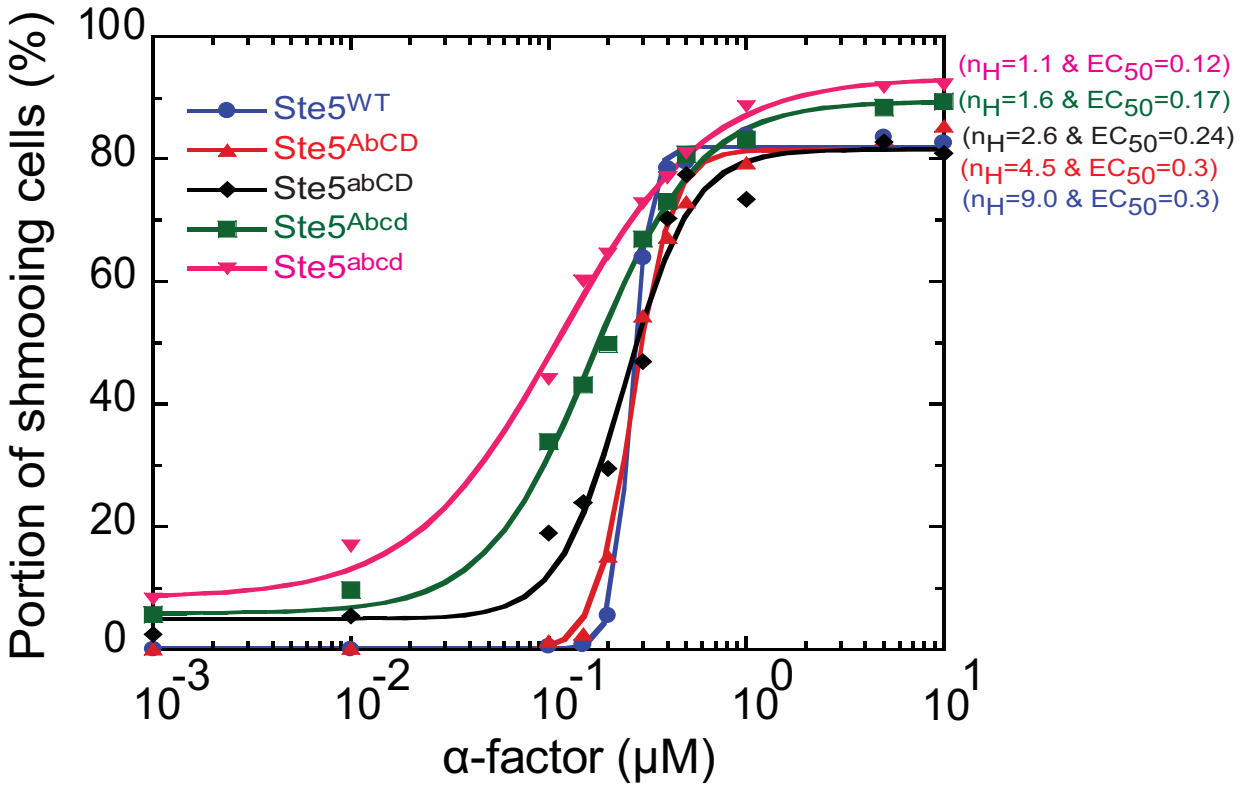
As predicted, the sigmoidal character of the response is robust to increasing the concentration of the phosphatase Ptc1.



As predicted, we observe a loss in the sigmoidal character of the response as we reduce the degree of local saturation by reducing the number of phosphosites on Ste5.



The sigmoidal character of the Fus3-Ste5 interaction determines the sigmoidal character of the fraction of shmooing cells.

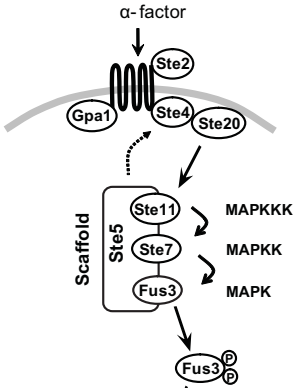


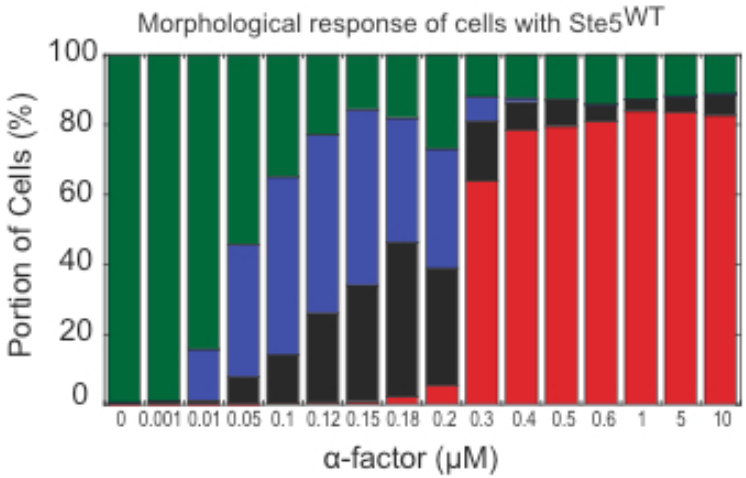
Decision to mate occurs early in the mating pathway.

The shmooing response to pheromone is highly ultrasensitive.

The scaffold Ste5 is an active component of the pathway.

Multiple phosphorylation sites on Ste5 and two-stage binding give an ultrasensitive response robust to the concentrations of the other components.

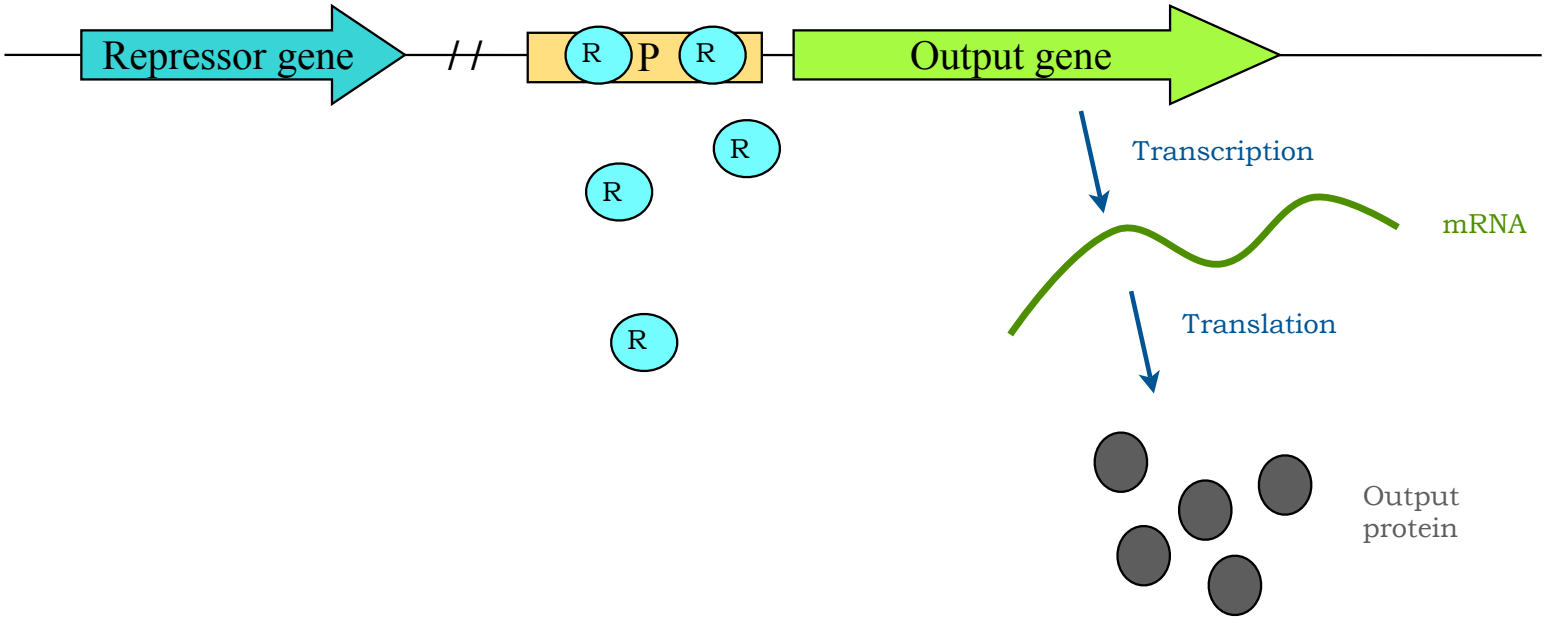




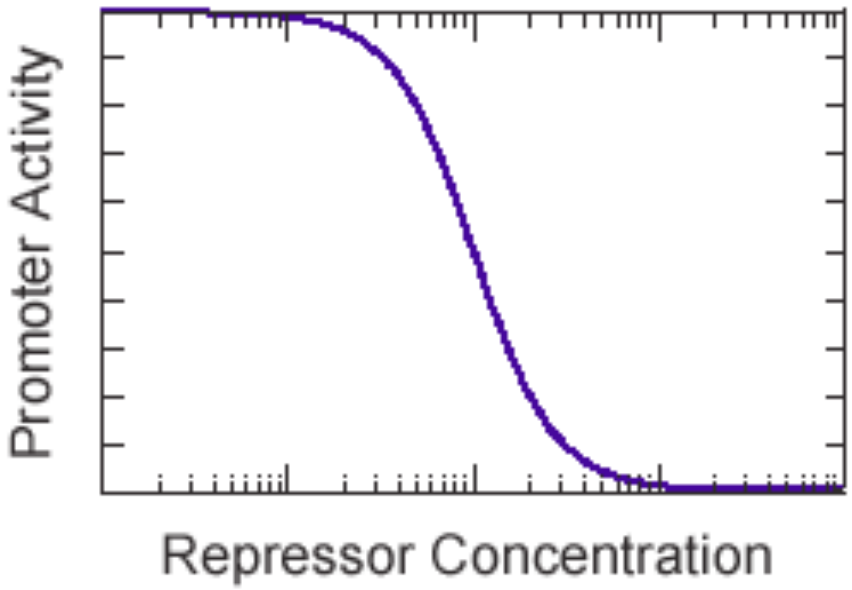
shmooing cells

Control of gene expression in bacteria

Gene expression: the rate of gene expression can be a sigmoidal function of the number of active transcriptional factors.



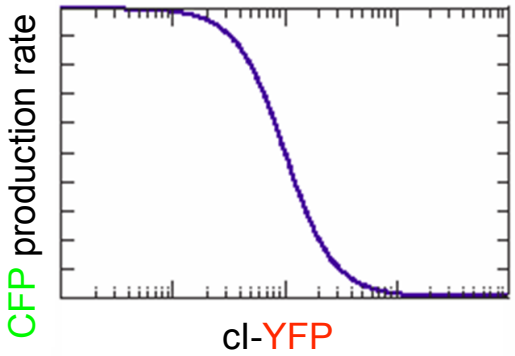
We expect less expression with more repressors.

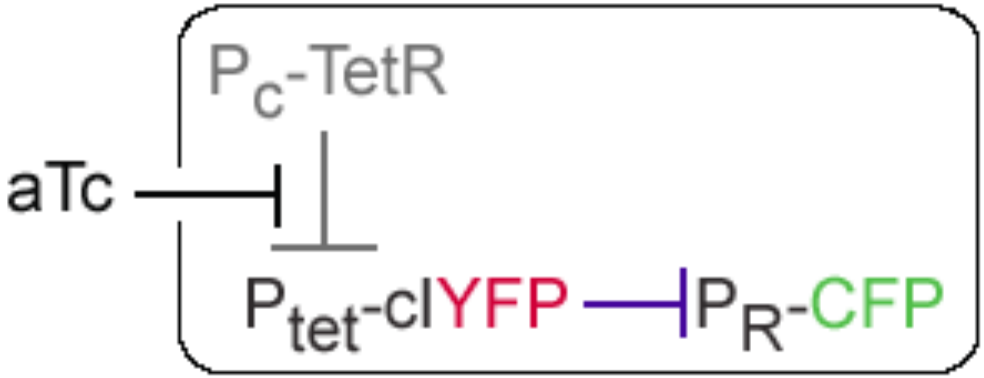


To measure the response, we create a synthetic network that allows simultaneous measurement of

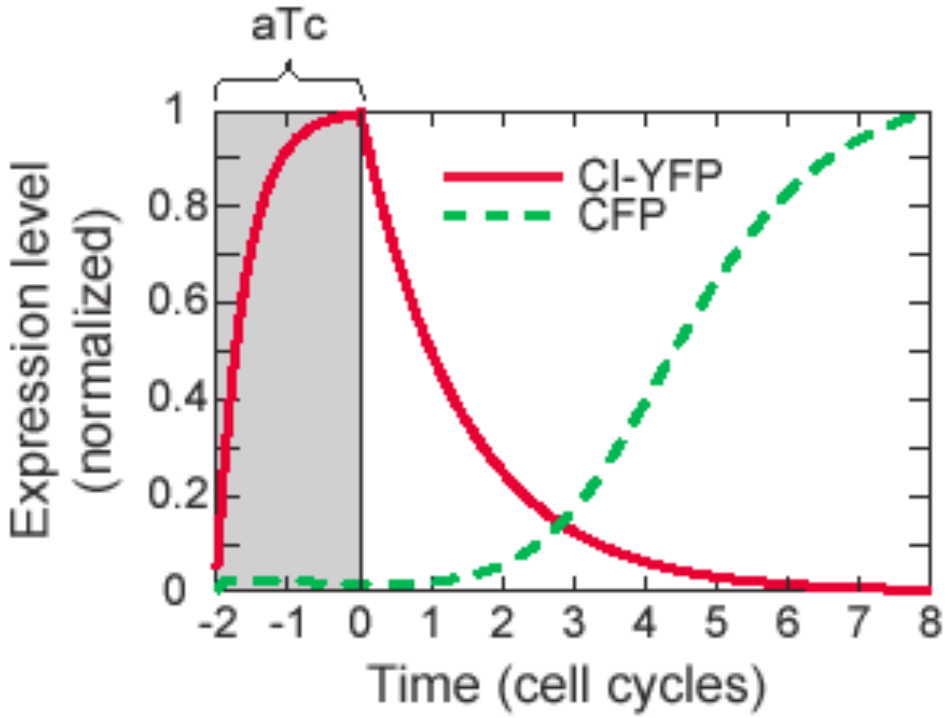
(i) the input signal: transcription factor (cI-YFP)

(ii) the output: the production rate of the downstream gene (CFP).

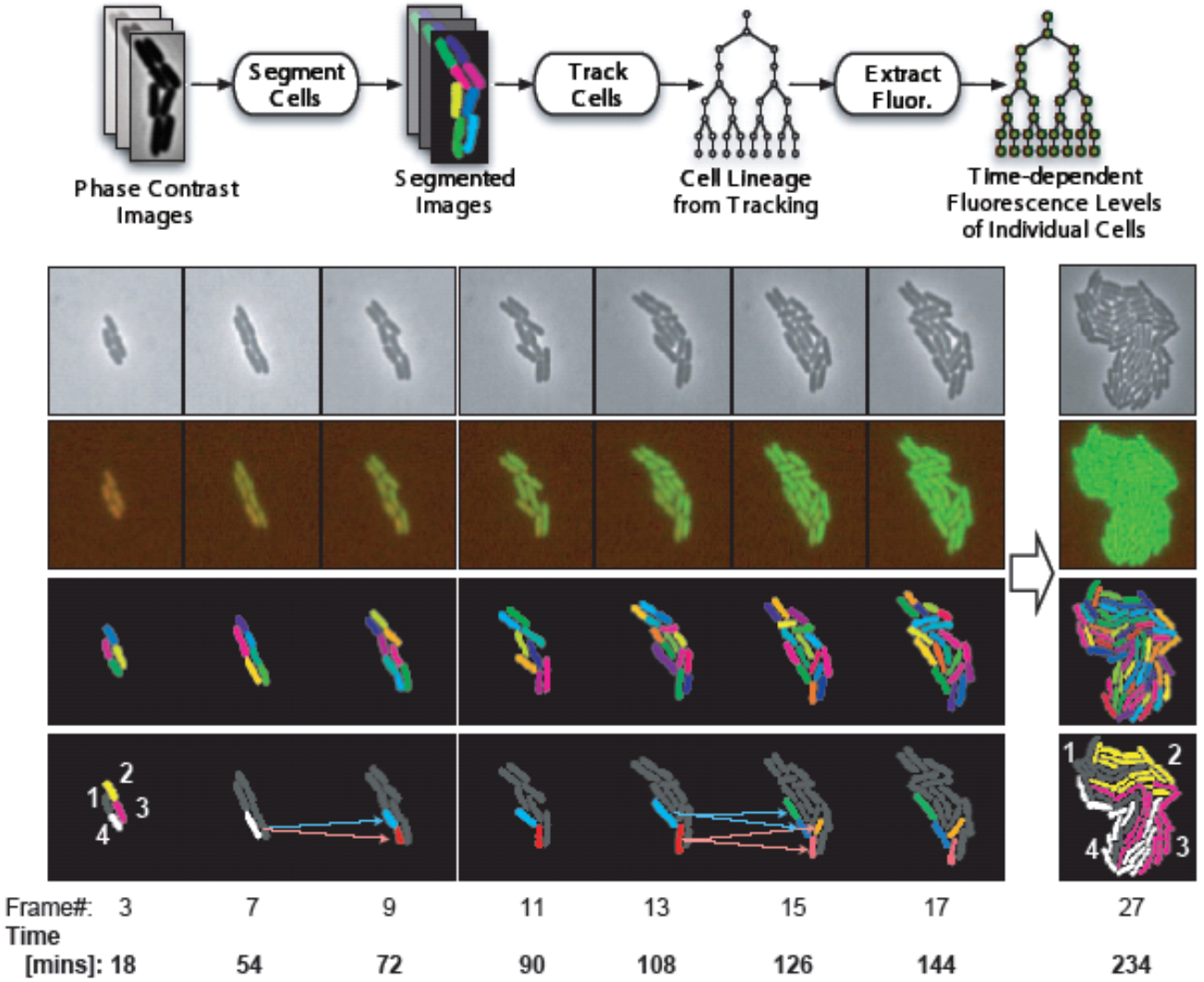


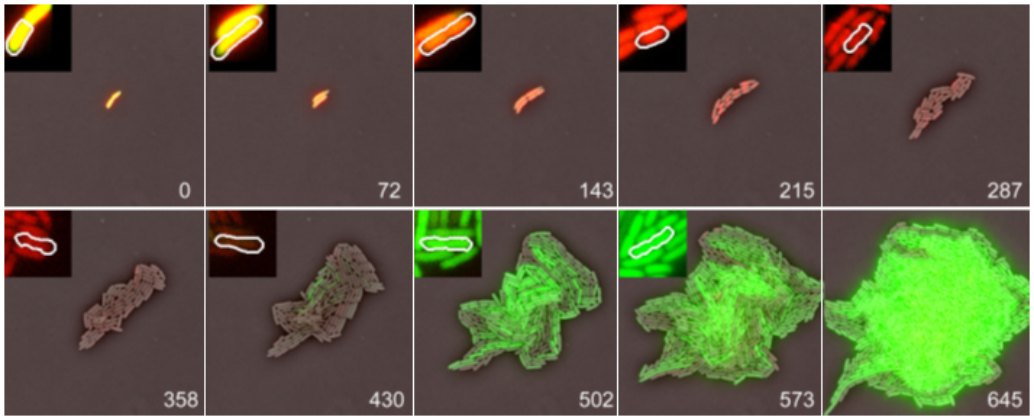


Experimental design: Fluorescently tagged repressor, in red, dilutes out as cells grow. The cell responds with new gene expression, in green, when the concentration of repressor falls sufficiently low.

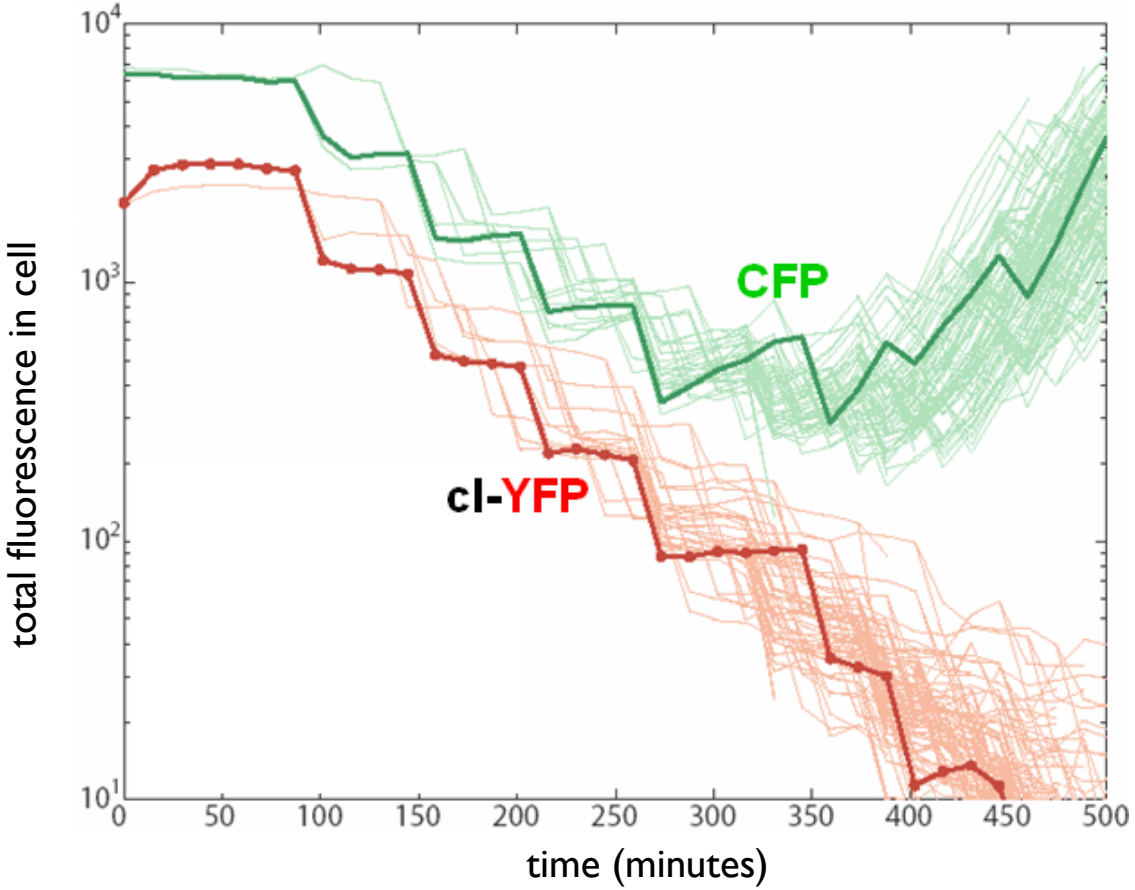


We developed the *Schnitzcells* software for automated image analysis and quantification of fluorescence levels.

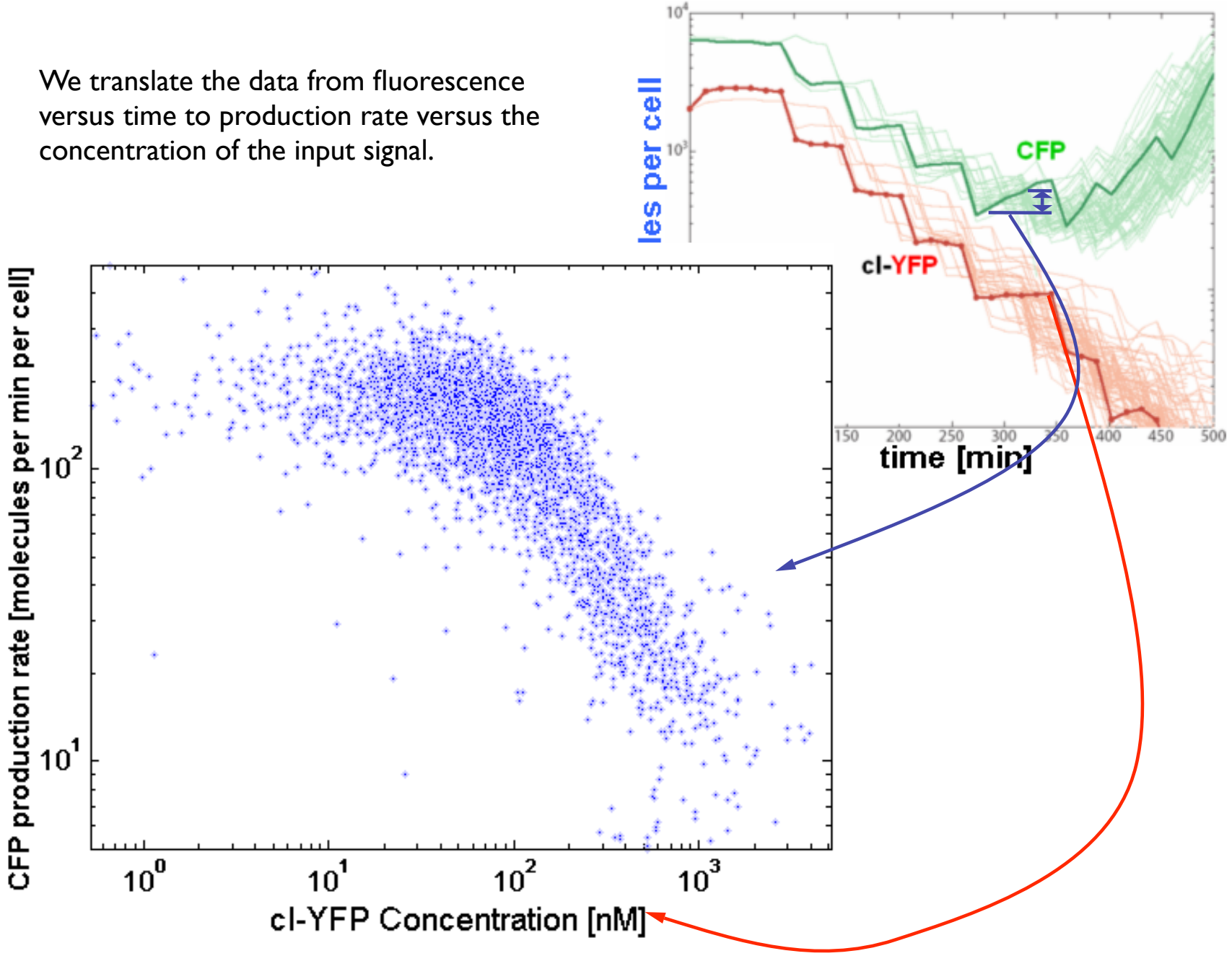




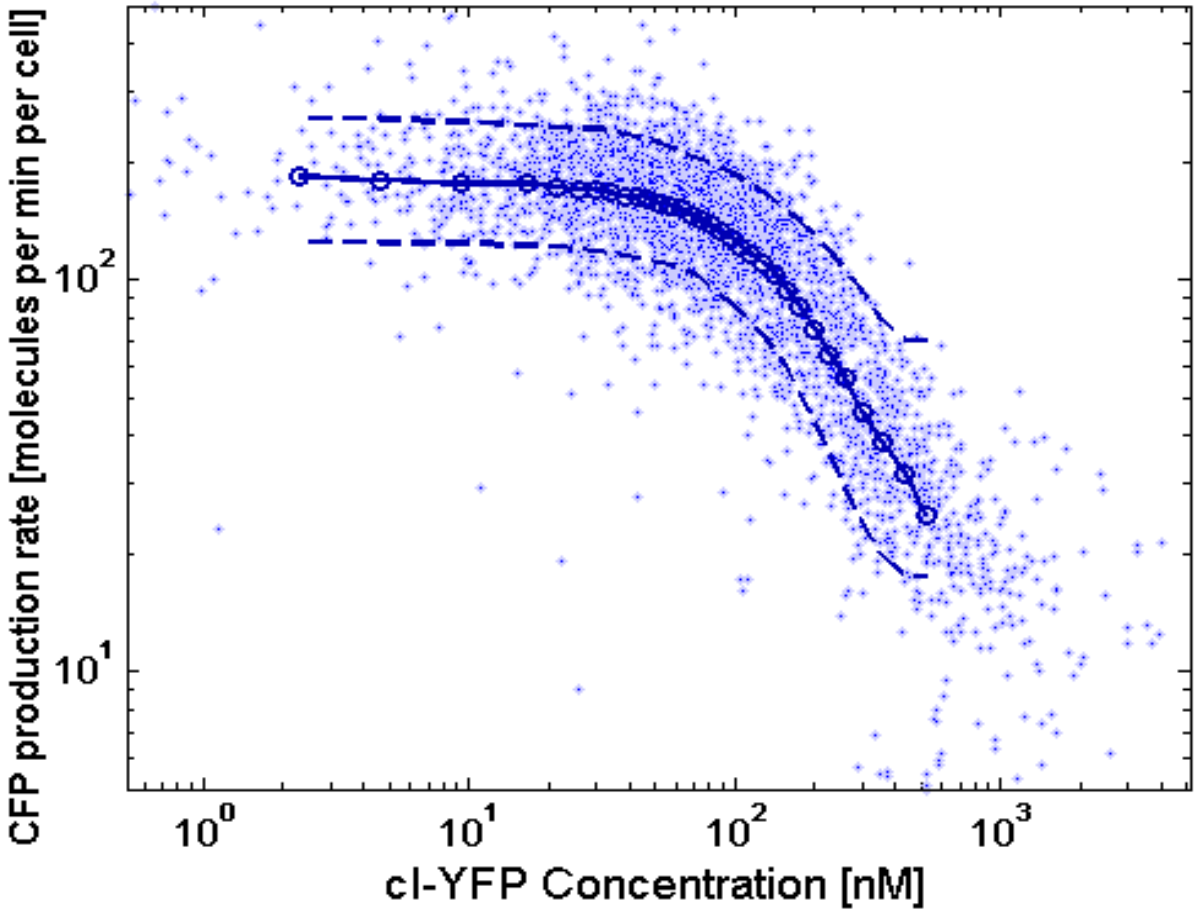
We can measure the fluorescence intensity levels of cI-YFP and of CFP in individual cell lineages.



We translate the data from fluorescence versus time to production rate versus the concentration of the input signal.

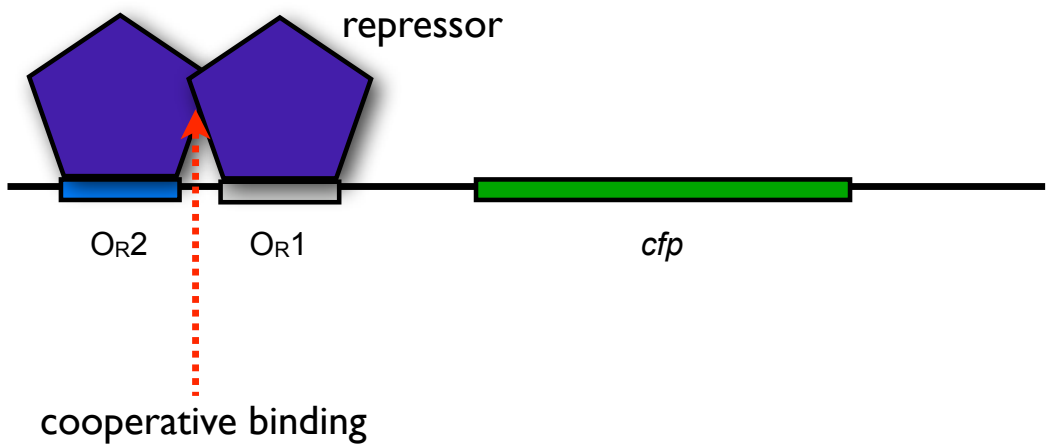


The mean input-output curve (with 95% confidence limits)

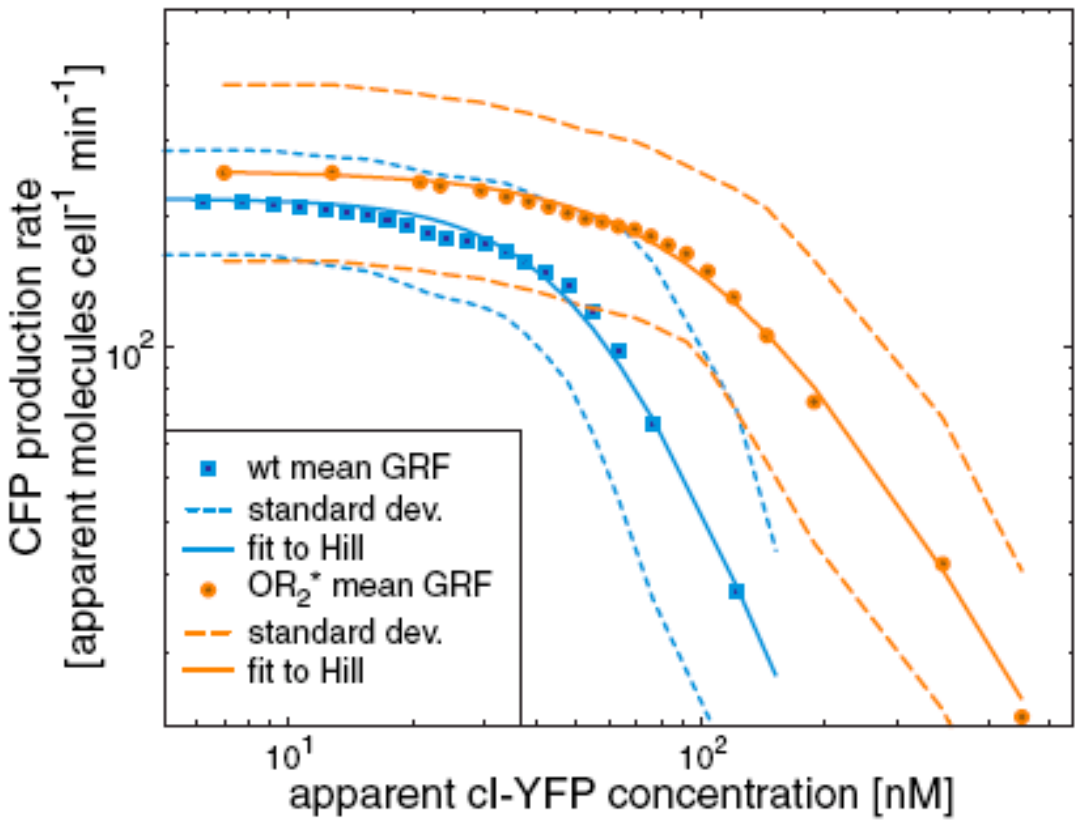


n_H is around 2

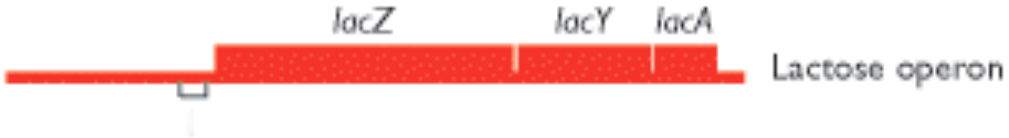
The promoter is activated cooperatively, or ultrasensitively, because two repressors bind to the DNA and each stabilizes the binding of the other.

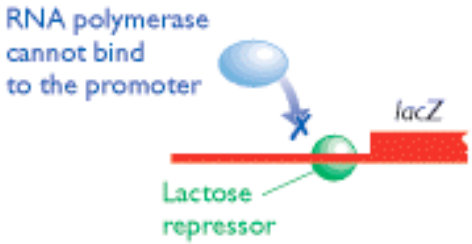


A mutation to the DNA that weakens the binding of one of the repressors reduces the degree of the sigmoidal character of the response.



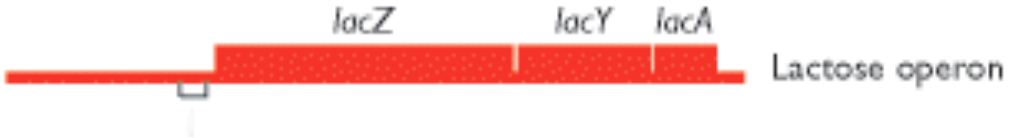
The same principles hold for induction of the *lac* operon: the input signal of sugar effectively dilutes out active repressor.

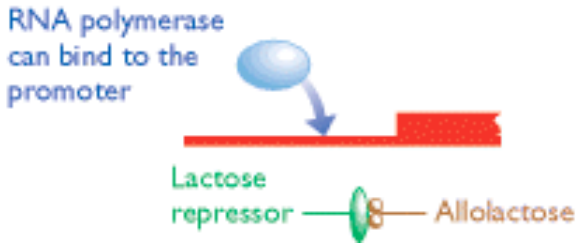




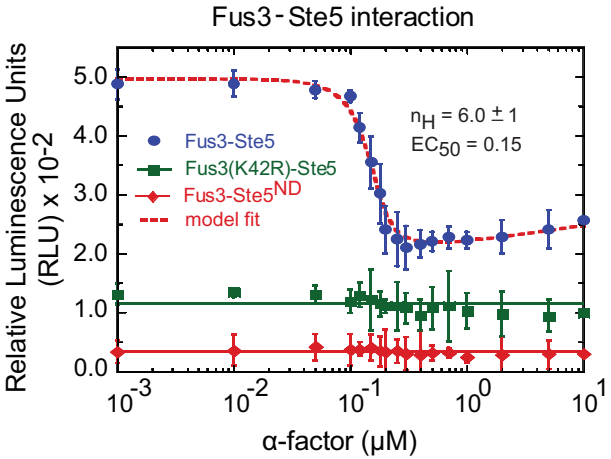
No transcription in the absence of lactose

The same principles hold for induction of the *lac* operon: the input signal of sugar effectively dilutes out active repressor.



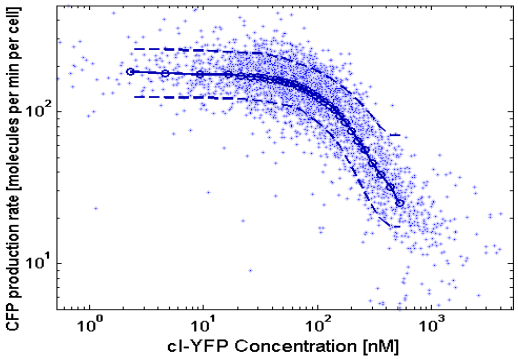


(Allo)lactose dilutes out active repressor and activates transcription

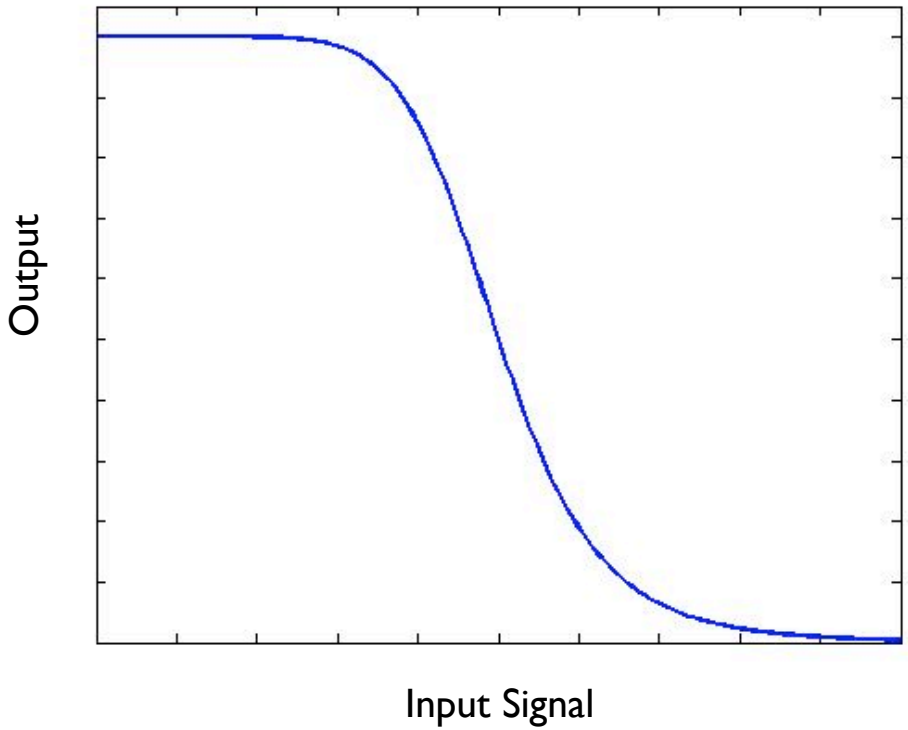


Ultrasensitivity is generated during signal transduction by a competition between a kinase and a phosphatase for a common substrate.

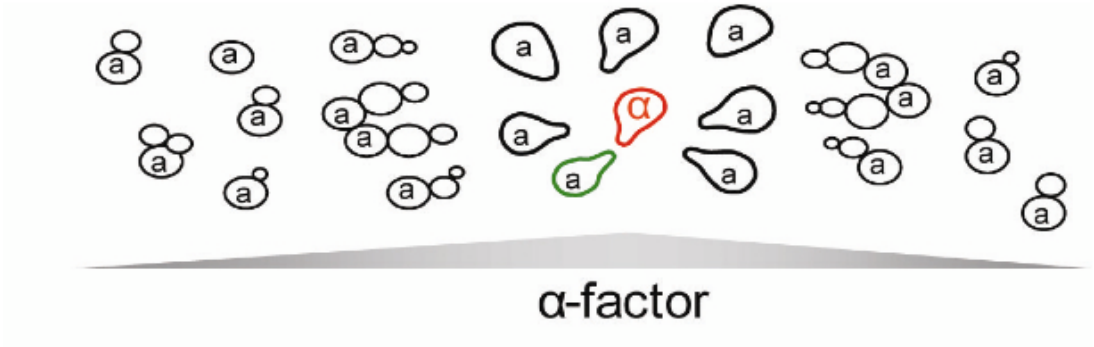
Ultrasensitivity is generated during gene expression by interactions between transcription factors at multiple binding sites at the promoter.



Why should both biochemical networks have sigmoidal responses?

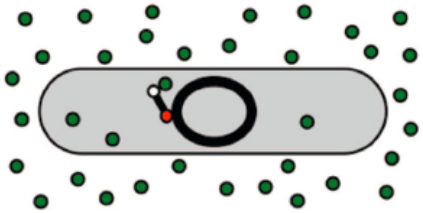


Both networks are responding to a similar challenge.



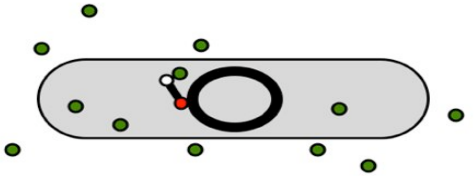
A yeast cell must decide whether to attempt to mate or not.

A bacterium must decide whether to express the enzymes to metabolize sugar or not.



environment rich in sugar

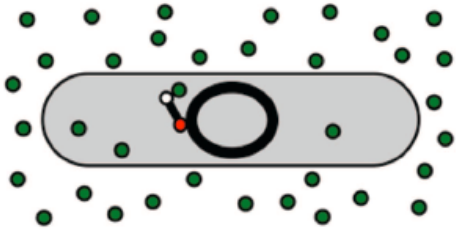
or



environment poor in sugar

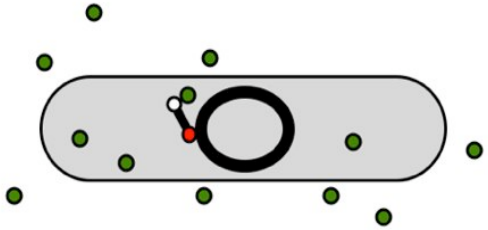
Consider if the bacterium is deciding in an environment that is in one of two states: a state high in sugar and a state low in sugar.

Stochasticity can make the intracellular state a poor predictor of the extracellular state.



high sugar
extracellular state

low sugar
extracellular state

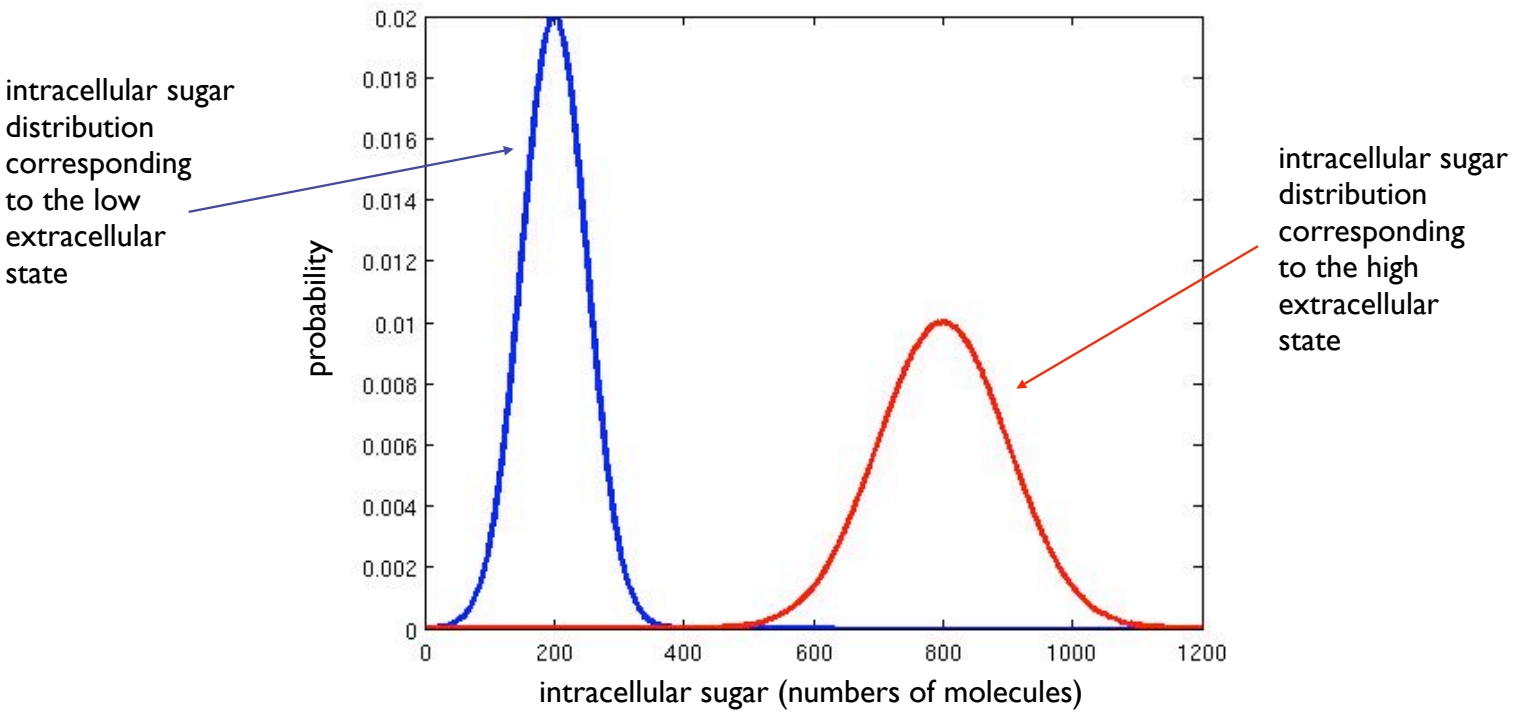


To be successful, a bacterium ought to respond to a

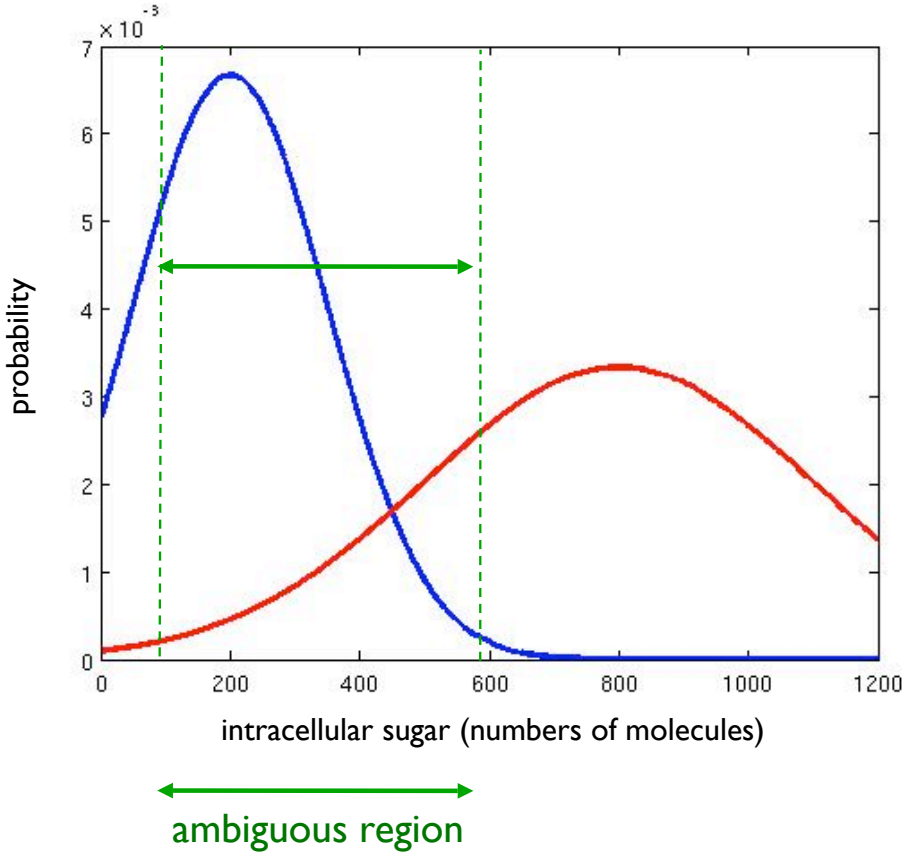
low extracellular sugar state → no expression

high extracellular sugar state → expression

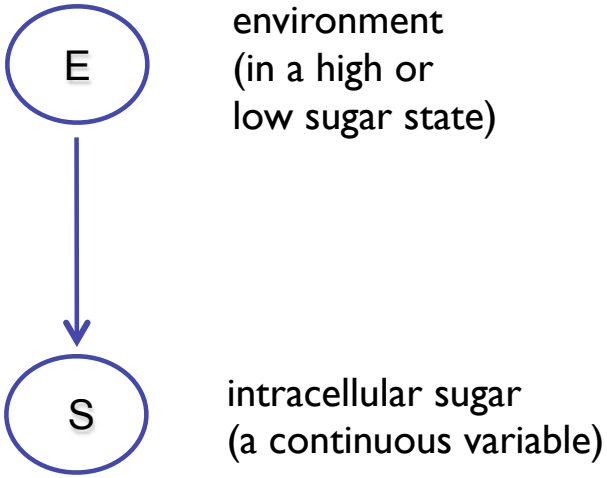
Distinguishing from intracellular sugar whether the extracellular environment is in the high (red) or low (blue) sugar state is mostly unambiguous if stochasticity is low.



With high large stochasticity, identifying the high or low extracellular state is no longer unambiguous.



Bayesian inference is a scheme to update prior belief with new data.



environment
(in a high or
low sugar state)

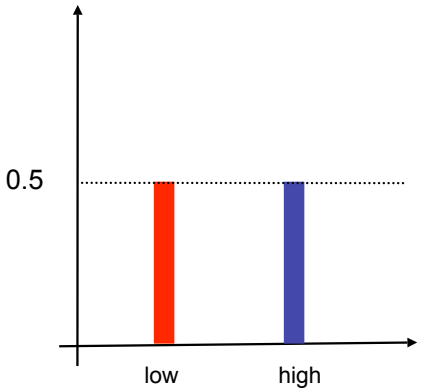
intracellular sugar
(a continuous variable)

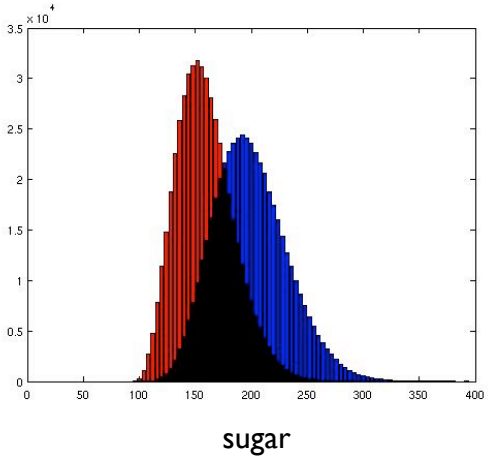
posterior probability

prior probability

$$P(E = \text{high} \mid S = s) = \frac{P(S = s \mid E = \text{high})P(E = \text{high})}{P(S = s)}$$

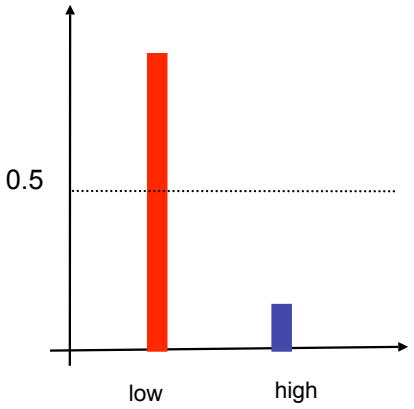
prior probability



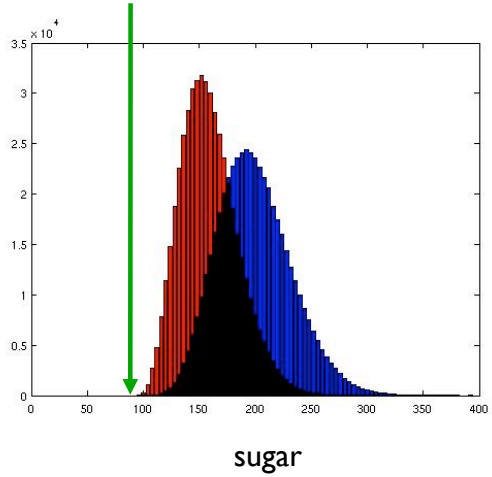


sugar distributions in the two states

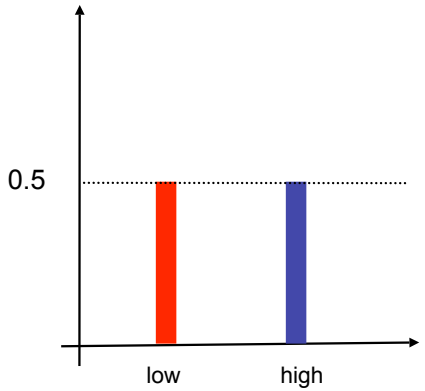
posterior probability

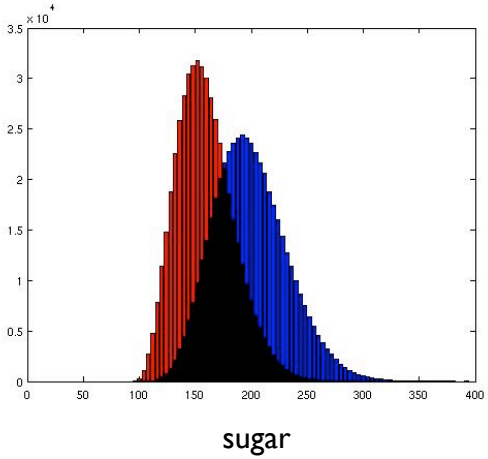


intracellular sugar

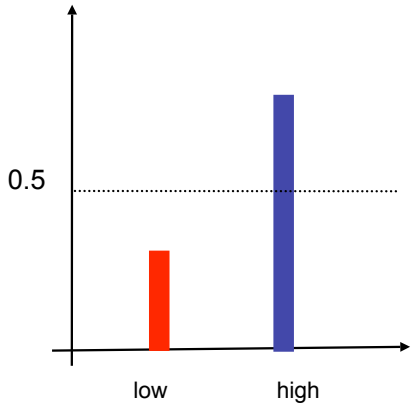


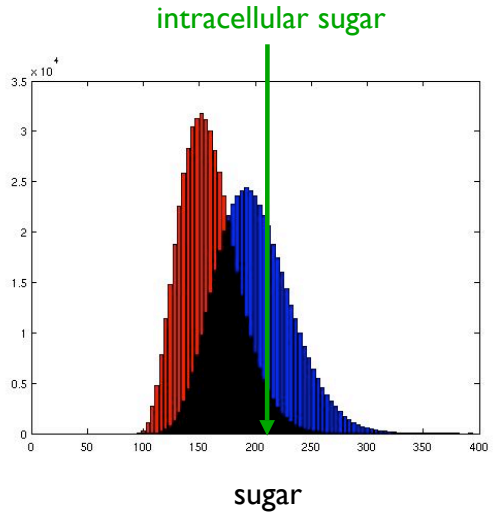
prior probability



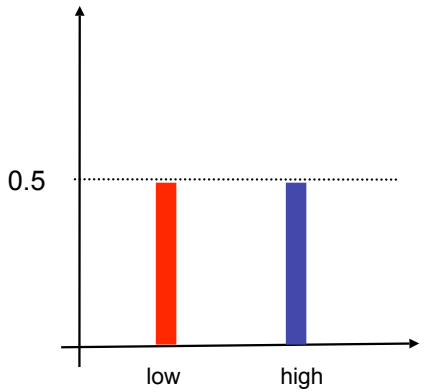


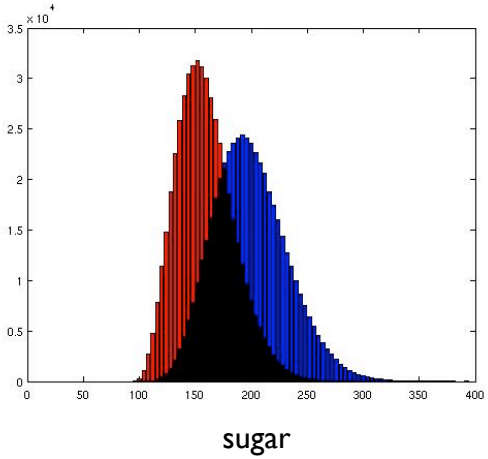
posterior probability



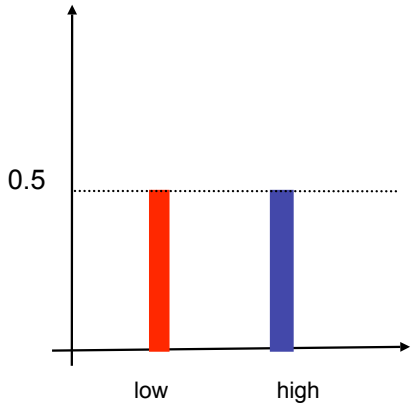


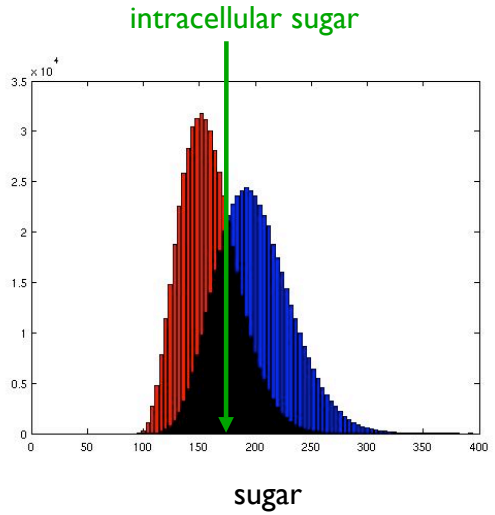
prior probability





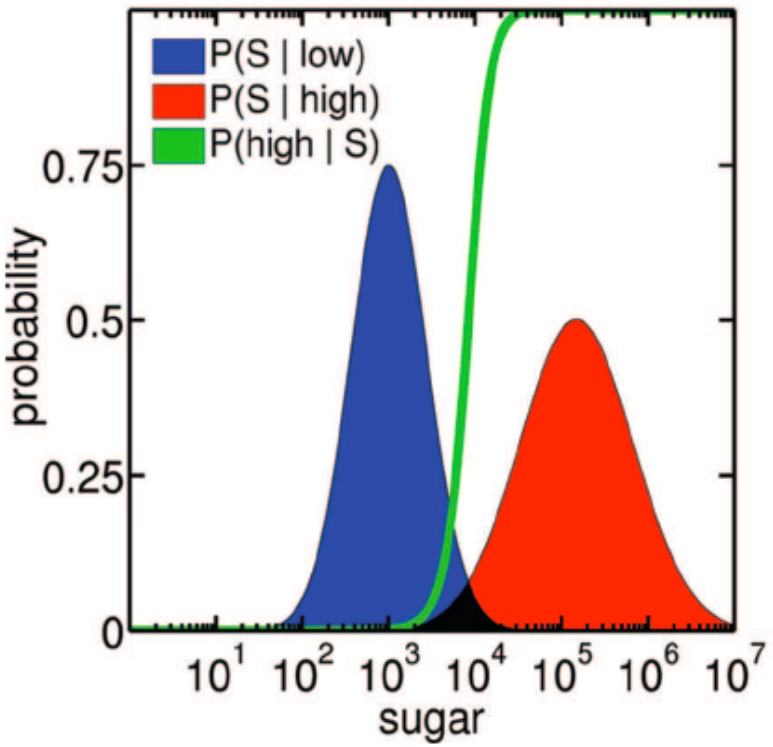
posterior probability



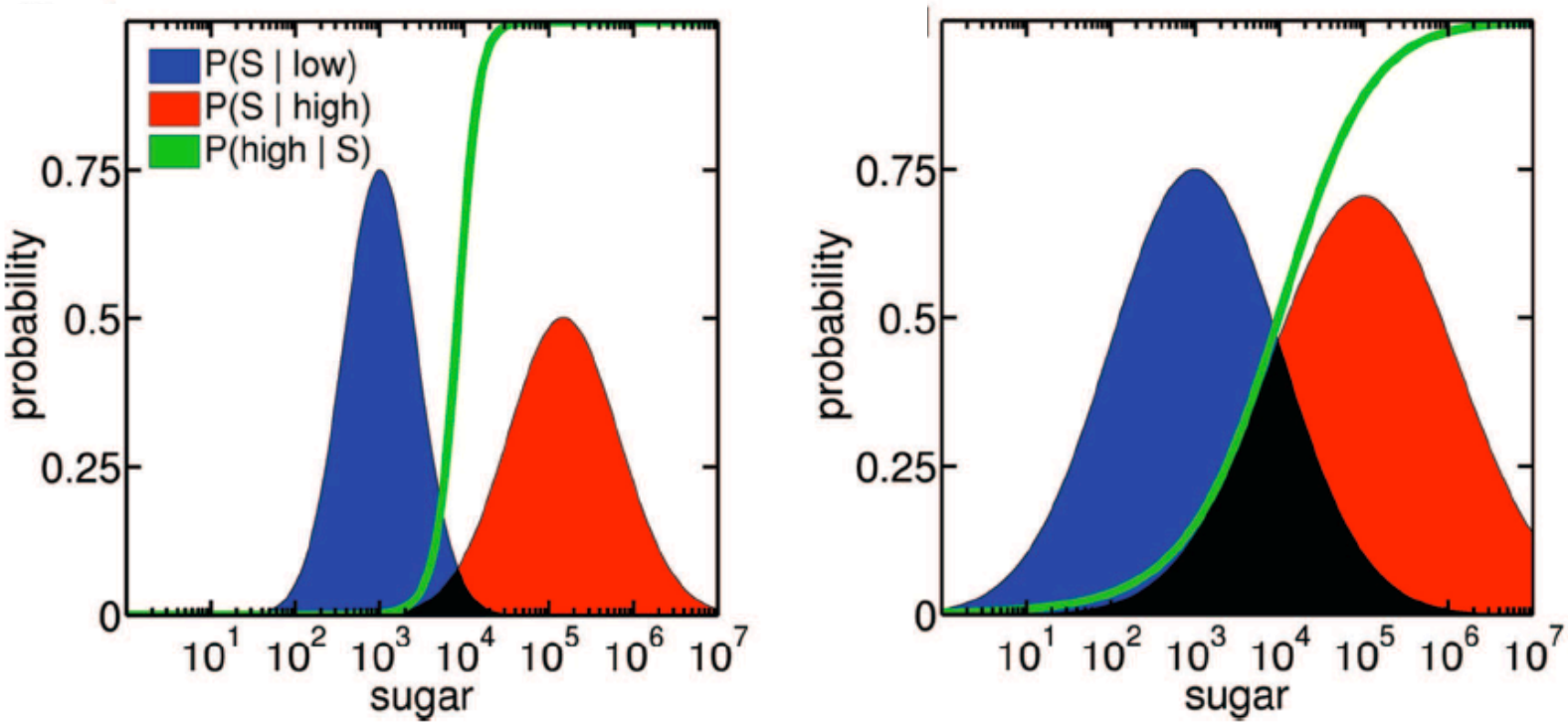


For a two-state classification problem, the posterior probability is often a sigmoidal curve. Cells may respond sigmoidally because they are inferring the probability of an environmental state.

posterior probability of high sugar state in the environment



We can understand why one biochemical network is more or less sigmoidal than another. The degree of overlap between the two distributions (one for each state of the extracellular environment) determines the sigmoidal character of the posterior probability curve.



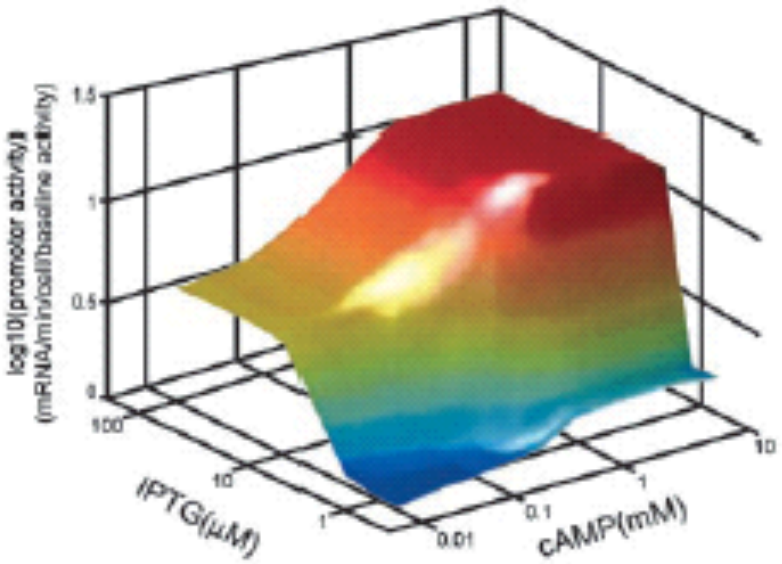
We can use the idea that cells are performing statistical inference to re-interpret experimental data.

Detailed map of a cis-regulatory input function

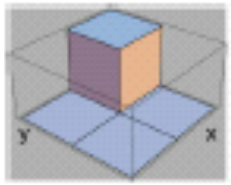
Y. Setty*†, A. E. Mayo*†, M. G. Surette‡, and U. Alon*†§

Departments of *Molecular Cell Biology and †Physics of Complex Systems, The Weizmann Institute of Science, Rehovot 76100, Israel; and ‡Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, AB, Canada T2N 4N1

promoter activity for the *lac* operon



expected output for an AND logic gate

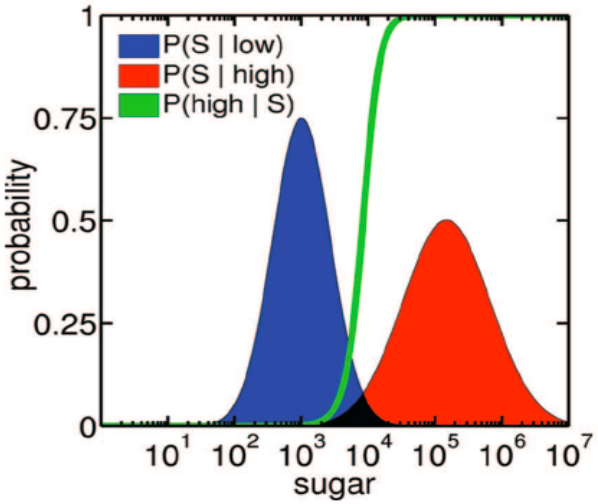


x AND y

high IPTG <-> high lactose
high cAMP <-> low glucose

Two-state classification problem:

given the two input distributions determine the probability of the high sugar state (green curve).

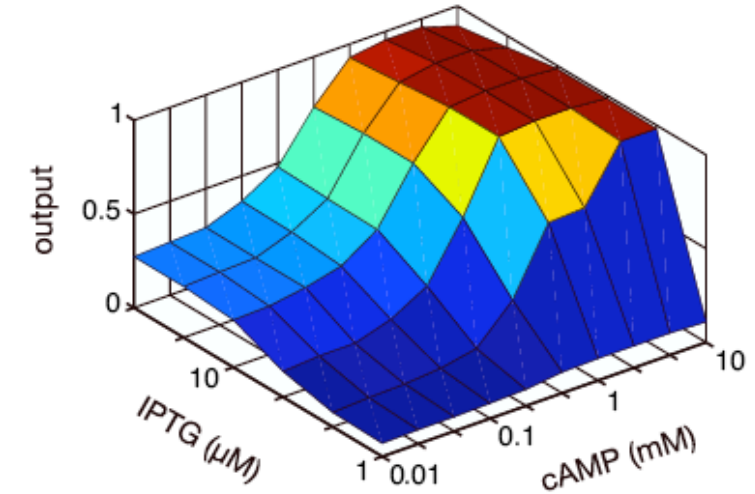


Inverse two-state classification problem:

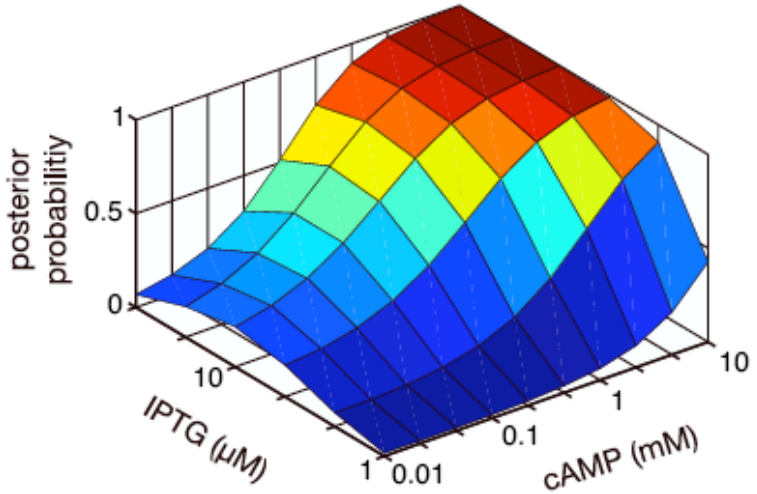
given the probability of the high sugar state (green curve) determine the two input distributions (red and blue distributions).

We fit a posterior distribution for the state high in lactose to the experimental data.

A posterior distribution for a two-state problem with the high and low states defined by two variables (IPTG, cAMP) fits the *lac* operon data.



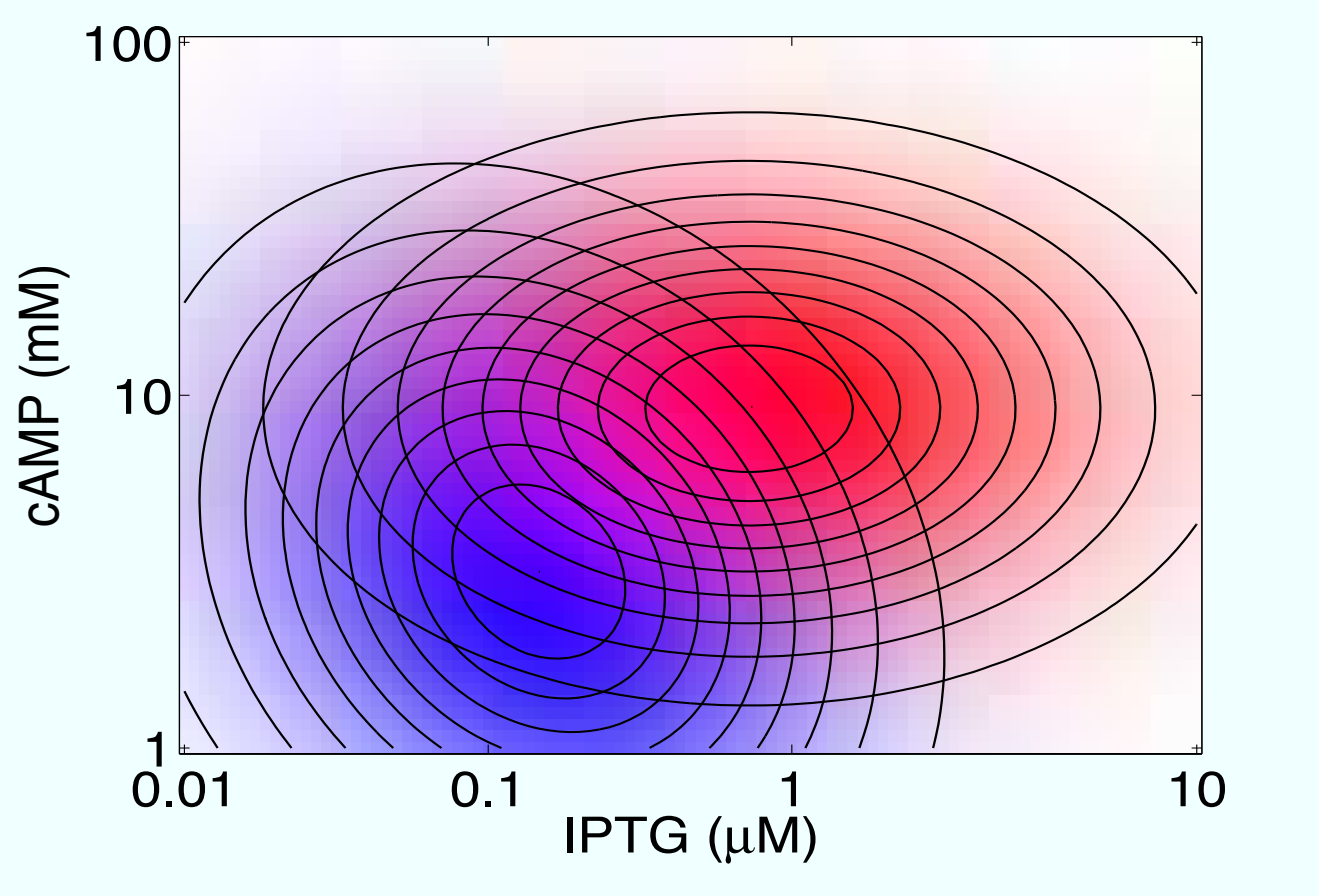
lac operon data



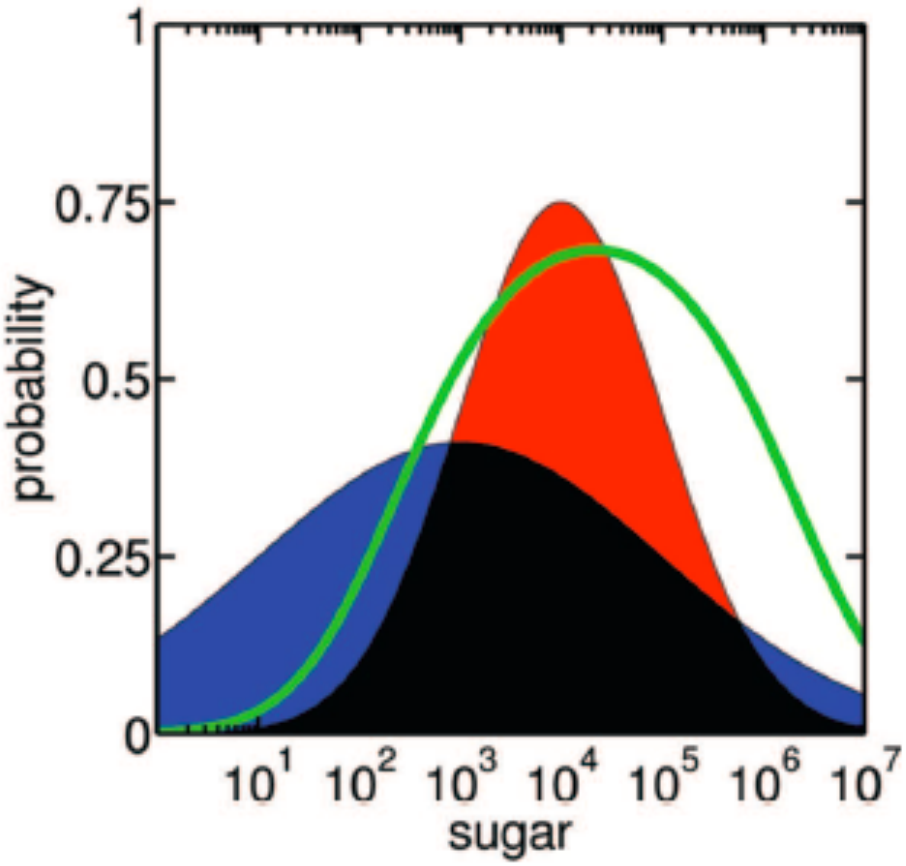
posterior probability of state high in lactose (and low in glucose)

We can estimate the statistics of the environment that natural selection has taught *E. coli* to expect.

Bivariate lognormal distributions for the two environmental states that generate a posterior probability that fits the promoter activity of the *lac* operon.



Aside: an inference module need not have a monotonic output.



Conclusions

1. Cells are decision-makers, but they must decide from sensing stochastic signals using stochastic biochemistry.

2. We can understand the types of responses cells make by considering that cells infer properties of their environment.

3. We need to study the responses of single cells and mimic as best as possible natural environments.

4. Acknowledgments

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