Chapter 6: Robust Signaling by bifunctional components

We begin our exploration of robustness by asking how a circuit made of noisy components can make precise computations. Such precision can be critical, for example, if the circuit needs to respond to stressful situations, and compute a response that is just right for the input stress. In this chapter we turn from transcription networks to the faster networks of protein-protein interactions that convey signals from the environment and culminate in the activation of transcription factors. These networks are called **signal transduction** pathways. Typically, the signal is sensed by a receptor (a sensor protein that sticks out of the cell membrane) that senses signals outside the cell and acts to chemically modify proteins inside the cell, thereby changing their activity. These proteins in turn can modify other proteins and so on. Finally, transcription factors get modified and activate genes that respond to the signals. These reactions take place on the scale of seconds to minutes. So far, we considered these pathways as instantaneous, and regarded them as the signals Sx and Sy that activate transcription factors. Now we look at more detail into these pathways.

6.1 Robust input-output curves

Let's define the robustness we seek. Suppose that a signal transduction circuit has an input signal s and an output, f(s), such as the level of activated transcription factor that activates genes to respond to the signal. The output as a function of input, f(s), is the circuit's **input-output curve**. A **non-robust** input-output curve depends on the concentrations of the proteins that make up the circuit (Fig 6.1). Since variations in protein concentration are an unavoidable property of biological matter, a non-robust f(s) means that different individual cells will show a different response to the same input signal. The input is inaccurately read by each cell.

In contrast, a **robust input-output curve** is insensitive to (and ideally completely independent of) variations in the levels of the proteins that make up the circuit. A robust input-output curve f(s) allows all cells to have the same output to a given input signal (Fig 1). Cells accurately perceive their environment.

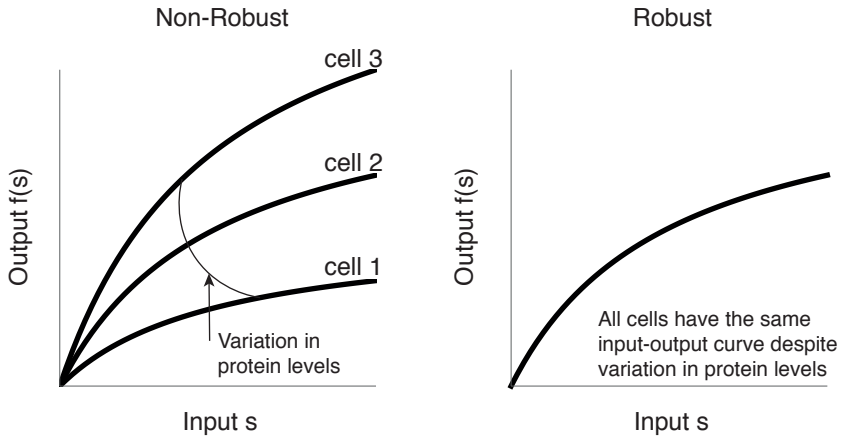

Figure 6.1

6.2 Simple signaling circuits cannot provide robust input-output relationships

Input-output robustness is difficult to achieve. To demonstrate this, let's consider a simple circuit (Fig 6.2) made of the typical components of signal transduction pathways. Signal is sensed by a receptor protein X that spans the cell membrane, and has a part that is outside the cell and a part that is inside the cell. On its outside part, the receptor detects the input signal. Signal causes the receptor to change conformation and hence transmit information to the part that is inside the cell. Information is then passed on to a messenger protein Y in the form of chemical modification, such as **phosphorylation**, in which a **phosphoryl group** PO4- is added to Y. The added group causes the messenger protein to change conformation into an active conformation. To pass this bit of information, inside the cell, X acts as a **kinase**, an enzyme that takes phosphoryl from

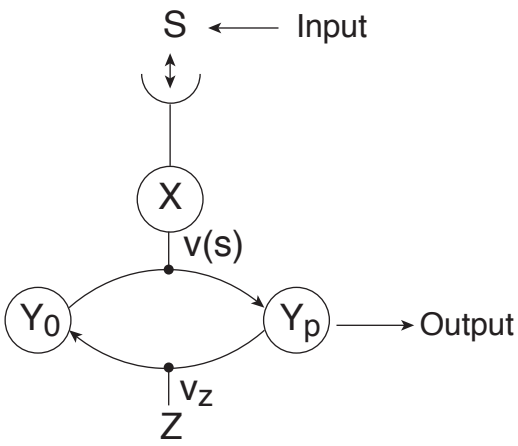

Figure 6.2

ATP adds it to protein Y (we say that X **phosphorylates** Y), at a rate that depends on the input signal. The phosphorylated messenger, Yp, is a transcription factor that binds promoters and activates output genes. Yp is the output of the circuit, the active transcription factor (Y*). To allow signaling to stop when the signal goes away, Yp is continually dephosphorylated (phosphoryl is removed into the cytoplasm in the form of inorganic phosphate Pi) by a **phosphatase** protein Z.

We will now solve the input-output curve of this circuit, to see that the curve depends on the concentrations of all of the three proteins that make up the circuit, X Y and Z. There is no input-output robustness.

The math is simple.

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Example X.1

Show that the simple design for a signal-transduction pathway has a non-robust input-output curve.

Receptor X phosphorylates Y at a rate v(s) that depends on input signal s, to produce Yp, the output of the circuit. The phosphorylation is removed from Yp by a phosphatase Z at rate vz. Let's compute the steady-state input-output curve Yp=f(s), and see how it depends on the concentrations of the proteins in the circuit, X Y and Z.

Y can either be phosphorylated, Yp, or not, Yo, such that the total concentration of Y protein is the sum of these two, Yt=Yo+Yp. We'll describe the dynamics of Yp using mass-action kinetics: dephosphorylation occurs when Yp and Z collide, at rate vz Z Yp, and phosphorylation occurs when X and Yo collide, at a rate v(s) X (Yt-Yp), where we used Yo=Yt-Yp. At steady-state, phosphorylation and de-phosphorylation must balance, dYp/dt=v(s)X(Yt-Yp)-vz Z Yp=0. Solving for Yp, we find that the input-output curve is an increasing function of the signal v(s)

$$Yp=f(s)=v(s)\ X\ Yt/(v(s)X+vz\ Z) \qquad (6.1)$$

This input-output curve depends on the levels of all three protein in the circuit: the receptor X, total messenger Yt and phosphatase Z (Fig 6.3). The more X and Y a cell has, and the less Z it has, the higher the input-output curve for a given signal s. Thus, each cell will respond differently to the same input signal. Since proteins typically vary in concentration by tens of percents, and this variation lasts an entire cell generation time, it will be common to have a two-fold difference in output between cells. There is no input-output robustness.

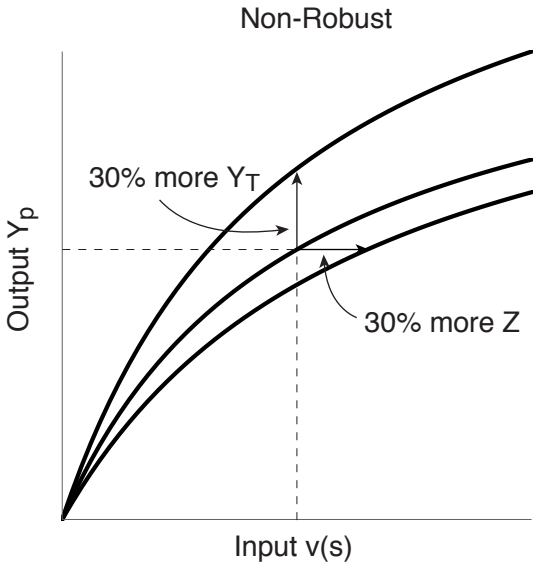

Figure 6.3

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In order to achieve input-output robustness, we need the protein levels to somehow cancel out in the expression for the input-output curve. Remarkably, bacterial **two-component systems** – a class of thousands of systems, each made of a specific receptor X and its dedicated messenger Y – are able to provide this robustness. At the heart of their design is a **bifunctional** component. The receptor X catalyzes two opposing reactions: phosphorylating Y at one catalytic site on the receptor, and de-phosphorylating Y at a different catalytic site (Fig 6.4). Thus, the opposing kinase and phosphatase activities **are rolled up into the same protein**, instead of being separated on two different proteins[1].

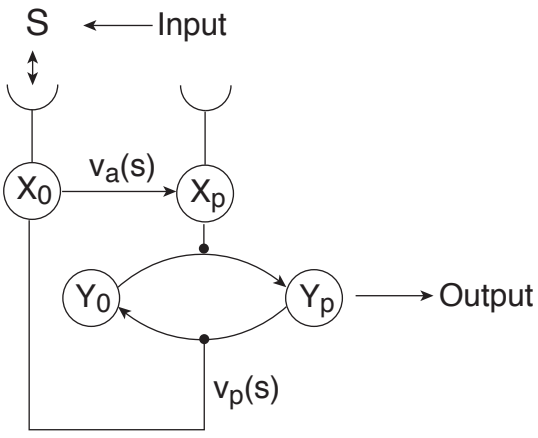

Figure 6.4

[1] To avoid confusion, note that every enzyme catalyzes a reaction and its reverse, with total flux determined by the concentrations of product and substrate. Bifunctional

A canonical example of this paradoxical design is the osmotic response system of *E coli*, EnvZ-OmpR. In this two-component system the receptor X is EnvZ, and the messenger Y is OmpR. When osmotic pressure is high, the receptor X phosphorylates Y to form Yp, a transcriptional activator of osmo-response genes (more precisely, X is an **autokinase** and a **phosphotransferase**, using ATP to phosphorylate itself and then transferring the phosphoryl to Y). Yp regulates genes such as transporters and enzymes that act to adjust the cell to the osmotic pressure in its environment. A robust input-output curve is crucial in this system because the response to osmotic pressure had better be accurate, to avoid the cell bursting or imploding.

Tom Silhavy and colleagues discovered that X carries out two antagonistic reactions (Hsing et al., 1998): it not only acts as a kinase that phosphorylates Y; it is also the phosphatase of Yp (Fig 6.4). It thus both adds and removes the chemical modification.

This bifunctionality, acting as both a kinase and phosphatase, was suggested by Russo and Silhavy (1993)) to enable robustness in the circuit. The intuitive reason is that a change in the concentration of the bifunctional protein X changes both phosphorylation and dephosphorylation rates by the same factor (Russo and Silhavy, 1993), thus canceling out the effect on the steady-state output Yp.

Robustness in this system was modeled mathematically and demonstrated experimentally by Eric Batchelor and Mark Goulian (2003)). Batchelor and Goulian experimentally changed the levels of the proteins X and Y in the circuit. They found that the output is robust despite large changes in the level of the proteins. For example, the output (the amount of Yp at a given input level of osmolarity) changed by less than 20% upon changes of 20-fold in the amount of total Y protein.

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enzymes do something more specific: they catalyze different reactions, at different catalytic sites. For example phosphorylation entails breaking down ATP, whereas dephosphorylation does not rebuild ATP (the reverse reaction) but instead moves the phosphoryl into the cytoplasm in the form of inorganic phosphate. Paradoxical enzymes are bifunctional enzymes that catalyze opposing reactions.

Guy Shinar et al. (2007) extended the theoretical analysis of this system, providing an analytical solution for its behavior. The special biochemical features of the receptor (autokinase, phosphotransferase, and phosphatase) combine to make Yp levels insensitive to variations in the concentrations of all proteins in the circuit—X and Y—and yet responsive to the input signal of the system.

It's fun to solve this system, in order to see how this cancelation comes about. The solution also uses a black-box trick that can be generalized to other systems.

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Example 6.2

Show that the bifunctional EnvZ-OmpR design has input-output robustness at steady-state with respect to fluctuations in the levels of proteins X and Y.

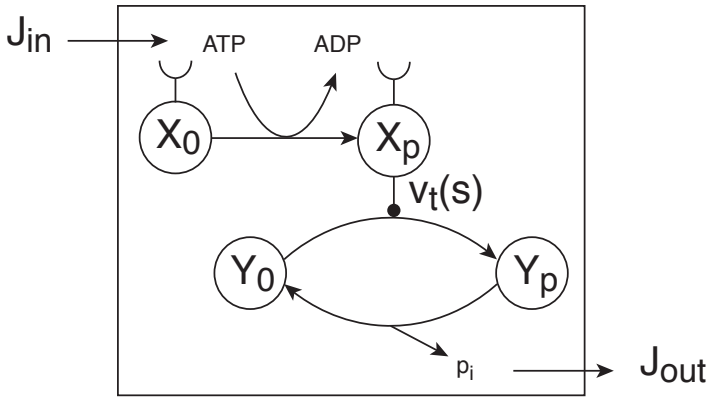

Figure 6.5

One way to solve this example is to write down seven mass-action equations for the system of Fig 6.5, and find their fixed point. This algebra is relegated to exercise XX. An easier way to obtain the input–output curve presents itself when we view the system as a **black box** that breaks down ATP and releases phosphoryl groups back to the cytoplasm (Fig 6.5). Consider the fluxes of phosphoryl into and out of the system. The influx, Jin, is equal to the rate of auto-phosphorylation in which the receptor takes phosphoryl from ATP and phosphorylates itself at a rate that depends on the input signal, va(s). Thus Jin=va(s) X. The outflux is the rate of dephosphorylation of Yp by the receptor, Jout=vp(s) X Yp, which releases the phosphoryl groups back into the cytoplasm as inorganic phosphate, Pi. At steady-state, influx and outflux must balance, Jin=Jout, otherwise the black box would fill up with phosphoryl groups. This means that

va(s) X = vp(s) X Yp (6.2)

Notice how X can be elegantly cancelled out from both sides of the equation (as long as X =/=0). We obtain a robust input-output curve that depends only on kinetic rate constants:

Yp= va(s)/vp(s) (6.3)

Importantly, the output Y_P *does not depend on the level of any of the proteins in the system.* The output is responsive to the input signal via the rate constants $v_a(s)$ and v_p(s). The mechanism thus shows a robust input–output relation (Fig. 2 *c*). QED

Input-output robustness is achieved by the coordinated effect of the biochemical details of this system. If the receptor was not bifunctional, and instead dephosphorylation was carried out by a separate phosphatase protein Z, the balance of phosphoryl influx and outflux would require that $X \sim ZY_P$. No cancellation of X is possible. This would result in a steady-state level $Y_P \sim X/Z$ that depends on the intracellular levels of both X and Z. Robustness would be lost.

Similarly, the two-step nature of the kinase is also essential for robustness. If the receptor directly transferred a phosphoryl group from ATP to Yo without first phosphorylating itself, the influx would depend on the concentration of the complex X·Yo. Balancing influx and outflux gives XYo~XYp. As a result, the output Y_P would be proportional to Yo and would thus depend on the total level of Y, Y_T , abolishing robustness.

In summary, robustness in the present mechanism seems to require the combined effects of multiple biochemical features. These features occur in the vast majority of the thousands of known two-component systems that respond to stress and environmental signals in diverse bacterial species (Goldberg et al., 2010; Capra and Laub, 2012). For example, *E coli* has about 30 different two-component systems and all but one have this bifunctional design. We will discuss the exceptional circuit, bacterial chemotaxis, in the next chapter.

For years, I thought that input-output robustness is impossible due to considerations of units. The output Yp has units of concentration. Units of concentration, in any

mechanism that I could imagine, come from the concentrations of the proteins in the circuit (as in the simple signal transduction circuit of Fig 6.2). So where do the units of concentration come from in the bifunctional mechanism? The answer is the intrinsic molecular rate constants. The bifunctional mechanism gives units of concentration by the ratio of a first order and a second order rate constant, va/vp (va has units of 1/time, and vp of 1/time/concentration). These intrinsic molecular rate constants are determined by the receptor structure, which is the same in all cells. These rate constants are therefore much more hard-wired (much less variable from cell to cell) than protein concentrations.

In fact, these intrinsic rate constants make the input-output curve even more elegant when the receptor does its two opposite functions according to Michaelis-Menten binding of the signal s. If the kinase rate va rises with binding (va~s/(k+s)) and the phosphatase rate vp decreases with binding (vp~k/k+s), the output Yp becomes linear in signal, Yp=va/vp~s/k. It's nice to have a linear undistorted readout of the input information.

But robustness always has its limits. In this system, robustness is lost if the total level of protein Y, denoted Y_T, falls below the expected Y_P level for a given input signal (Fig 6.6).

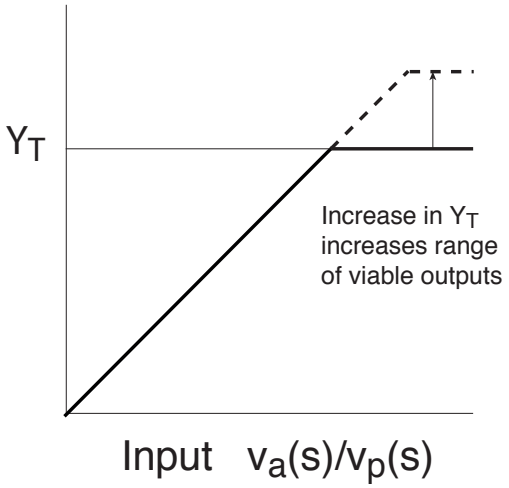

Figure 6.6

In this case, there is not enough Y protein to reach the Y_P value given by the input-output curve Eq. 6.3. When this happens, analysis of the model (see exercise 6.5) shows that all of the Y molecules are phosphorylated, and $Y_P = Y_T$. All of X is also phosphorylated Xp=Xt. Hence, the system is maxed out and no longer responds to the input signal (mathematically speaking, unphosphorylated X is zero and cannot be canceled out in the black box equation). We conclude that both robustness and responsiveness to the signal require that total Y levels Y_T exceeds a certain threshold, given by the maximal desired output level Y_P in the expected physiological conditions (exercise autoregulation XX).

There is also call for caution. When studying models such as this, we need some healthy skepticism. We need to watch out for additional reactions in the cell, perhaps too weak to be experimentally detected, that can potentially ruin robustness. To explore this possibility, we can add to the model every possible reaction arrow and assign to it a small rate ϵ. For example, we can add spontaneous dephosphorylation of Y_P (Yp→Yo +Pi without help from X). This spontaneous reaction is known to occur in the EnvZ/OmpR system on a timescale of minutes which is much slower that the other reactions which take seconds or less (thus ϵ is ~seconds/minutes~0.01). We can also add spontaneous de-phosphorylation of X_P, reverse phosphotransfer in which Xp goes back to Xo, effects of ATP and ADP as cofactors, and so on. Some of these possibilities are explored in exercises 6.7 and 6.8 The upshot is that the effect on robustness of these additional reactions is either nonexistent, or is small (in the sense that the relative shift in the input-output curve due to protein fluctuations is of order ϵ).

Remarks on the Black Box Approach.

To analyze the robustness mechanism, we considered the system as a black box that breaks down ATP. The black box approach can be used more generally, to suggest a wider class of systems that can show ro

The black box argument depends only on balancing two reactions, the entry of phosphoryl and the exit of phosphoryl from the box. This leaves us with freedom to add any number of reactions inside the box (as long as a stable steady-state is reached and no new entry or exit points are added). For example, we can introduce a cascade of phosphotransfer events (as occurs in many bacterial signaling systems), Fig 6.7, and robustness of Yp is still maintained.

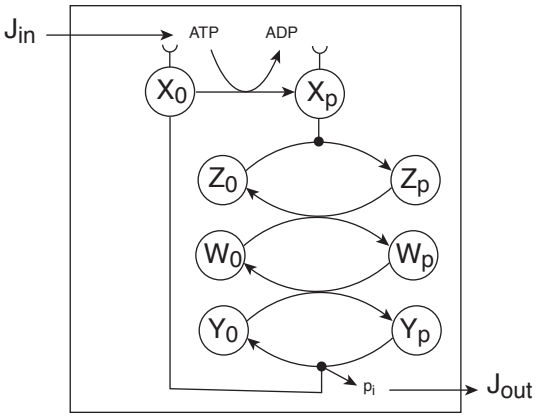

Figure 6.7

The black box also points to system characteristics that rule out such robustness. The black box suggests that robustness of the present type cannot generally occur if there is more than one reaction that introduces (or removes) phosphoryl groups into the system. If two different influxes J_i and J'_i exist, they generally cannot be canceled out with J_o (in the sense of Eq 6.2 above), leading to a loss of robustness. Of course if such secondary leaks or inputs are small, of order ϵ, robustness is only lost to order ϵ.

Not all bacterial signaling systems show the hallmarks of the present mechanism. Important examples include bacterial chemotaxis, which we will study in the next chapter, and sporulation. Apparently, these

signaling systems do not need robust input–output relations, but instead rely on cell–cell variation in their output in order to provide a wider range of solutions to a given situation. A robust input-output mechanism should perhaps be expected only in signaling systems in which there is a sufficiently heavy fitness penalty if the input–output relation is not precise.

Bifunctional components provide robustness to diverse circuits

The principle of robustness by bifunctional enzymes that catalyze opposite reactions (also called **paradoxical enzymes**) applies to other systems and organisms(Hart review). In each case, a paradoxical enzyme is at the core of the mechanism, and additional biochemical features combine to allow robustness. Examples include nitrogen regulation in E coli, explored in exercise 6.12, in which an enzyme modifies and de-modifies a key metabolic enzyme in nitrogen control. A paradoxical enzyme in human cells makes and breaks an allosteric regulator of the main nutritional pathway, glycolysis. Paradoxical enzymes also operate in tissue-level circuits. For example, a paradoxical enzyme called Piezzo1 makes epithelial cells proliferate and also die according to pressure signals that indicate if there are too few or too many cells in the tissue (Gudipaty nature 2017 doi: 10.1038/nature21407). In the immune system, T-cells secrete a signal ml]olecule called IL-2 that makes them both proliferate and die, helping to maintain a desired concentration of T-cells (Hart Cell 2014). Theoretical analysis of antagonistically bifunctional enzymes led to a mathematical theorem that can predict which components of a complicated biochemical reaction system might be robust (Shinar and Feinberg, 2010; Karp et al., 2012).

It is possible that many more paradoxical enzymes exist than are currently known. The same effects can be produced by two mono-functional enzymes that carry out opposite reactions, by having the two enzymes work together only when they form a complex, or are held together by a scaffold protein.

In summary, robustness is a major concern for the precision of biological circuits. It is also a central issue in engineering, where circuits need to work precisely despite variations in components due to imprecise fabrication of, for example, resistors and transistors. In biological signaling circuits, bifunctional components can provide robust input-output curves despite unavoidable fluctuations in the levels of the proteins that make up the circuit. The robustness is due to a combination of specific biochemical details, and thus provides a systems-level meaning to biochemical details that may otherwise appear arbitrarily complicated.

Further reading:

E. Bachelor and M. Goulian. Robustness and the cycle of phosphorylation and dephosphorylation in a two-component regulatory system. Proc Natl Acad Sci U S A. 2003 Jan 21;100(2):691-6.

Shinar G, Milo R, Martínez MR, Alon U. Input output robustness in simple bacterial signaling systems. Proc Natl Acad Sci U S A. 2007 Dec 11;104(50):19931-5.

Hart Y, Alon U. The utility of paradoxical components in biological circuits. Mol Cell. 2013 Jan 24;49(2):213-21.

Shinar G, Feinberg M. Structural sources of robustness in biochemical reaction networks. Science. 2010 Mar 12;327(5971):1389-91.

Exercises:

6.1 Mass action for the non-robust circuit: Solve the mass-action kinetics of the three protein signaling circuit of Fig 6.2, taking into account the complexes of the reactants. Show that the input-output curve is not robust.

Solution: Let's assume that ATP binds X very strongly, a realistic assumption for most signaling systems, so that free X is always bound to ATP. The reactions are [XATP]+[Yo]←koff1,kon1→[XATP Yo]-v(s)→ [X]+[Yp] +[ADP] and [Z]+[Yp]←kon2,koff2→[ZYp]-vz→[Z]+[Yo]+[Pi]. Thus the rate of change of the complex [XATP Yo] is a balance of binding, unbinding and catalysis

d[XATP Yo]/dt=[XATP][Yo] kon1 - [XATP Yo](v(s)+koff1).

At steady state, d/dt=0, and [XATP Yo]=[XATP][Yo](kon1/(va(s)+koff1). Typically for enzymes koff1>>va(s), and so [XATPYo]=[XATP][Yo]/K1 where K1=kon1/koff1. Hence phosphorylation rate is va(s) [XATP Yo]=[XATP][Yo]va(s)/K1. Dephosphorylation rate, from an analogous calculation, is [Z][Yp] vz/K2 where K2= kon2/koff2. Balancing the two, and using [Yo]+[Yp]=[Yt], we obtain [Yp]=[XATP][Yt] va(s)/K1 /(va(s) [XATP] [Yt]/K1 +vz [Z]/K2). This input-output curve is non-robust because it depends on the concentrations of the proteins in the system.

6.2 Correlated expression can increase robustness: One way to partially address the non-robustness for the simple three protein signaling circuit of Fig 6.2 is to make fluctuations in protein levels correlated, by putting Y and Z on the same operon. Discuss why this can improve robustness, but not make the circuit absolutely robust.

Two-component mechanism

6.3 The seven mass-action equations for the two-component circuit: A detailed two-component mechanism includes ATP and ADP and the complexes of the reactants. The reactions are:

Xo+T<-k1',k1->A-va(s)->Xp+D

Xp+Yo<-k2',k2->B-vt->Yp+Xo

A+Yp<-k3',k3->C-vp->Yo+A+Pi

Where T ad D are ATP and ADP, A is the complex [X T], B is [XpYo] and C is [A Yp], and Pi is inorganic phosphate.

a) Write down the mass action kinetic equations and conservation laws.
b) Solve for the steady states.
c) Show that one steady-state describes the case where there is enough Y protein for the desired output, and the other applies when there is not enough Y.

Solution:

a) There are two conservation laws, for total X Xt=X+Xp+A+B+C,

and total Y

Yt=Yp+Yo+B+C.

The seven mass action equations are

dXo/dt=-k1 Xo T +k1’ A+vt B

dXp/dt=-k2 Xp Yo+va(s) A+k2’B

dYo/dt=k2’B +vp C –k2 Yo Xp

dYp/dt=-k3 A Yp +k3’ C +vt B

dA/dt=Xo T k1 – (va(s) +k1’) A

dB/dt=Xp Yo k2-(vt+k2’) B

dC/dt=A Yp k3- (vp+k3’) C

b)Solving these at steady-state shows two solutions. In one solution, solution 1, Xp=Xt, Yp=Yt and all other concentrations are equal to zero. In the other solution, solution 2, Yp*=(va(s)/vp) (k3’+vp)/k3, which is robust because no protein concentrations appear in it.

c)Stability analysis shows that solution 2 is the only stable solution when Yp*<Yt. If Yp*>Yt, solution 1 is the only stable solution.

6.4 A more precise black box calculation: The calculation in the main text did not take into account complexes of the proteins. Repeat the black box calculation taking into account the complexes.

Solution: Jout=vp [X Yp]. Let’s compute the concentration of the complex [XYp]. The complex is formed by the binding of X to Yp, and lost when the constituents dissociate or when the de-phosphorylation reaction takes place: d[XYp]/dt= k3 X Yp – (k3’+vp)[X Yp]. Thus, at steady-state, the concentration of the complex is proportional to the product of its component concentrations:[X Yp]=k3/(k3’+vp)X Yp, which yields Jout=vp k3/(k3’+vp)X Yp . At steady-state, Jin=Jout, otherwise the black box would fill up with phosphoryl groups. This means that va(s) X = vp k3/(k3’+vp)X Yp. We therefore obtain a robust input-output curve that depends only on kinetic rate constants:

[**] Yp=f(s)= (k3'+vp) va(s)/k3 vp

Exercise 6.5: **Loss of robustness when Yt is too low**:

(i) Do a black box calculation when when Yt falls below the value of Yp expected form the robust mechanism. Show that the system enters a saturated state in which all of Y and all of X are phosphorylated. Why is this state a non-signaling state?

(ii) What happens when total levels of receptor X becomes very low? Does this place any limitations on signaling?

6.6: **Limits to linearity of the output curve:** In the robust mechanism, when va(s)~s/(k+s) and vp(s)~k/(k+s) both depend on Michalis-Menten binding of the signal s, the output curve can be linear in s: Yp=va/vp~s/k. But every linearity must have its limits. Explain what processes might break linearity at very high signal levels.

6.7: **Reverse phosphotransfer from Yp does not affect robustness:** Add a reverse phosphotransfer reaction to the two-component model, in which Yp+Xo→Xp+Yo. Use the black box approach to argue that this this additional does not affect robustness or the steady-state output.

Exercise 6.8: **Spontaneous dephosphorylation leads to small loss of robustness:** In the EnvZ-OmpR circuit, Yp can be spontaneously dephosphorylated without the action of X. The half-life of Yp due to this reaction is ~90 minutes, compared to half-life of Yp of seconds due to dephosphorylation catalyzed by X.

a) Write an equation for Yp dynamics assuming the two-component mechanism also has a reaction of spontaneous dephosphorylation at rate ϵ.
b) Use the black box approach to calculate the steady-state level of Yp.
c) Explain why robustness is only lost to order ϵ.

Exercise 6.9: **Energy consumption.** The EnvZ-OmpR system continually uses up ATP, even for constant input signals.

(a) Discuss why constant energy expenditure might be useful in this signaling circuit.
(b) Suppose there are X molecules of X per cell that use 100 ATP/second. Estimate the fraction of the bacteria's ATP consumption that goes to running this circuit.

Exercise 6.10: **Dynamics of the robust mechanism.** Suppose the input signal rises in a step from level s1 to level s2.

(a) Compute the dynamics of the robust mechanism, Yp(t). Assume low signals so that most of X and Y are unphosphorylated.

Answer (partial) : Low signal means : Yo~Yt, Xo~Xt.

dYp/dt=vt XpYo-vp XoYp~vt Xp Yt-vp Xt Yp

dXp/dt=Xo v(s)-vt XpYo~Xt v(s)-vt Xp Yt

Adding these equation yields

d(Xp+Yp)/dt= Xt v(s) –vp Xt Yp

The dynamics are a sum of exponentials of time with eigenvalues lambda1= -vp Xt and lambda2=-vt Yt.

(b)What is the response time?

Dynamics are dominated by the smaller eignevalue. Since Yt>Xt (Xt~100/cell, Yt~3500 /cell) and vt>vp (phosphotransfer is much faster than dephosphorylation), lambda2>lambda1. The smaller eigenvalue is lamba 1~vp Xt. Hence the response time is ln(2)/vp Xt.

(c) Is the response time robust to variations in X? in Y?

Exercise XX: stability of the two fixed points in the robust mechanism- **transcritical bifurcation.**

Exercise 6.11: **Positive autoregulation and robust input-output relations**: In many two component systems, the output transcription factor Yp is a transcriptional activator of its own gene and the gene for the receptor X (often both genes are on the same operon). If signaling output Yp is robust to total X and Y levels, what can be the role of positive autoregulation? (Hint: consider strong input signals, REF).

Bifunctional enzymes and robustness in other systems

The theme of robustness based on paradoxically bifunctional enzymes occurs in diverse systems. In each system, antagonistic bifunctionality provides a robust input-output relationship, but each system studied so far has also shown a different combination of features that generate this robustness.

Exercise 6.12: Paradoxical control in *E coli* carbon/nitrogen balance: E coli bacteria must balance their uptake of carbon and nitrogen. The key enzyme that assimilates nitrogen (in ammonia) into biomass is the enzyme GlnA, made of 12 identical subunits (dodecamer), which produces the amino acid glutamine, Q. The dilemma is that Q is made at the expense of a carbon backbone that is a key metabolite in the tricarboxylic acid (TCA) cycle, alpha-ketoglutarate, denoted K. Making too much Q depletes K; therefore, the Q/K ratio is important and stays nearly constant in a wide range of conditions (Senior, 1975; Brauer et al., 2006). **The** robustness of the Q/K ratio depends on a bifunctional enzyme AT/AR which both activates and deactivates GlnA by

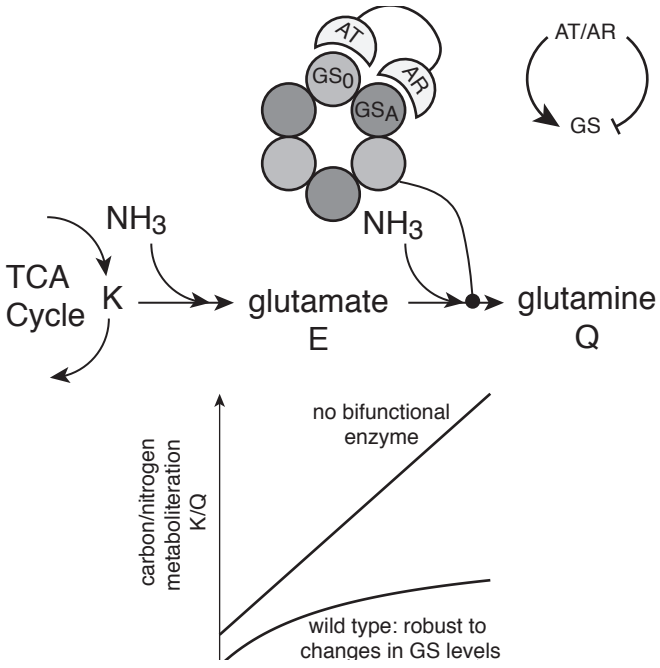

Figure 6.8

removing and adding an adenylyl modification. The twist is that AT/AR can bind two GlnA subunits in the same dodecamer (Fig 6.8), and hence shows a strong avidity effect: if it binds one subunit, it is likely to bind both. Thus, a ternary complex T in which the bifunctional enzyme binds two substrates, one modified and the other unmodified, carries out most of the reactions (Fig 6.8). The rates of adenylation and de-adenylation are equal at steady state: $v_1(Q,K)\ T = v_2(Q,K)\ T$. Here, the specific rates of the two reactions carried out by the bifunctional enzyme are v_1 and v_2, and both depend on Q and K.

(a) How can this design lead to a robust Q/K ratio?
(b) Explain the results of an experiment in which GS levels are experimentally controlled by expressing it form a plasmid, and the Q/K ratio is measured in wild-type cells and in cells deleted for the gene for AT/AR? (Fig 6.8b)
(c) Propose experiments to test the hypothesis that the bifunctionality of AT/AR is causal for robustness. Use the fact that the two reactions are carried out by different parts of the enzyme AT/AR, and that mutants are available that knock out one or the other function. (Hart et al., 2011a).

Exercise 6.13: **Coherent bifunctionality as a robust linear amplifier**

So far we discussed bifunctional enzymes that carry out two opposing reactions. What happens when a bifunctional enzyme carries out two reactions which go towards the same goal, such as increasing production and decreasing removal of a modification, and thus both acting to increase the level of the modification? Suppose enzyme X modifies protein Y with rate v+(s), and removes the modification with rate v-(s). Both rates are Michaelis-Menten, and are controlled by binding of a signal molecule s to the enzyme X. Production increases with s, so that v+(s)=a s/(k+s), whereas removal decreases with s, v-(s)=b k/(k+s). Assume that most of Y is unmodified, Yp<<Yt. What is the output (level of Yp) as a function of s?

Solution: dYp/dt=v+(s) X Yo+v-(s)X Yp, Yo=~Yt. At steady-state, the output is linear is signal:

Yp=Yt v+(s)/v-(s)=Yt (s/k+s)/(k/k+s)=Yt (a/b) s/k

(i) Explain why this can be called a **robust linear amplifier**?
The output Yp is linear in the signal s, and does not depend on (is robust to) the level of the bifunctional enzyme X.

(ii) What might be the limits of linearity for very high and very low levels of s?

(iii) What happens of the rates v+ and v- are Hill functions of s with the same k and n?

(iv) In the liver, the body stores glucose in a branched polymer called glycogen, an important part of the body's nutrient reserve. A hormone signal for starvation leads to the coordinated phosphorylation of two enzymes that make and break glycogen into glucose and back. Read about the mammalian glycogen system and model it using the concept of coherent bifunctionality.