Practical Systems Biology

Peter S. Swain
peter.swain@ed.ac.uk
Biological Sciences, University of Edinburgh

Contents

1 Overview

These notes are based on lectures given to M.Sc. students in the School of Biological Sciences at the University of Edinburgh.

In Secs. 2 and 3, we start with the fundamentals of mathematical modelling, both of signal transduction and of gene expression. These sections are necessarily the most complex mathematically, but throughout we will illustrate the techniques by developing a model of a signalling pathway (Fig. 1). We then turn to the effects of positive and negative feedback. Positive feedback can generate bistability and is used by cells to differentiate irreversibly (Sec. 4). Negative feedback can cause oscillations and drives biological rhythms (Sec. 5).

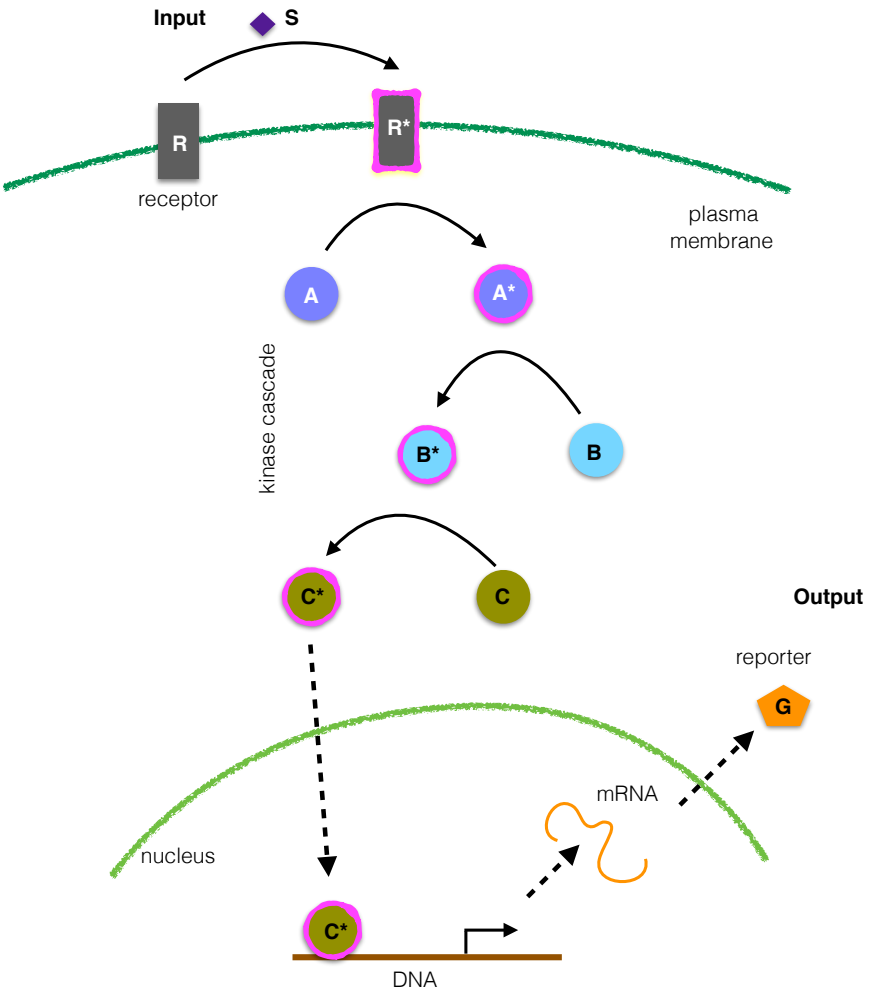


Figure 1: An idealized model of a eukaryotic signalling pathway: an input, ligand S, activates receptors at the plasma membrane (activation is shown in purple), which in turn activate a cascade of kinases. The last kinase in the cascade, C, enters the nucleus once activated and enables expression of a reporter gene. The protein-product of this gene, G, is the system's output.

2 Modelling biochemical reactions

2.1 Chemical rate equations

Consider two reactions: the first occurs when an A and a B molecule come together, react, and form a C molecule; the second occurs when a C molecule dissociates back into an A and a B molecule. For example, the A molecule could be a receptor on the cell membrane and the B molecule could be an extracellular ligand. These two molecules come together reversibly to form a receptor-ligand complex, C, which can signal intracellularly. Let the rate at which a pair of A and B molecules associate into a C molecule be $\tilde{f}$ (measured in inverse seconds) and the rate at which a C molecule dissociates be $\tilde{b}$ (also measured in inverse seconds), then:

$$\mathrm{A} + \mathrm{B} \underset{\tilde{b}}{\overset{\tilde{f}}{\rightleftharpoons}} \mathrm{C}$$

The association rate, $\tilde{f}$, is determined by two times: the time taken by a molecule of A and a molecule of B to find each other by diffusion, t_{diff}, and the time taken for the two molecules to react once in physical proximity, t_{reac}. We can write

$$\tilde{f} = (t_{\mathrm{diff}} + t_{\mathrm{reac}})^{-1} . \tag{2.1}$$

We wish to describe how the number of C molecules, N_C, changes with time. Over a small interval of time dt, the association and dissociation reactions will both occur (we will include stochastic effects later). The number of pairs of A and B molecules is $N_A N_B$, and $\tilde{f} dt N_A N_B$ of these pairs will associate over a time dt. The number of C molecules that dissociate over dt is $\tilde{b} dt N_C$. Therefore, the number of C molecules at a time $t + dt$ is the number of C molecules at time t plus the number gained in association reactions and minus the number lost in dissociation reactions:

$$N_C(t + dt) = N_C(t) + \tilde{f} dt N_A N_B - \tilde{b} dt N_C. \tag{2.2}$$

Taking the limit of dt going to zero, we have

$$\frac{dN_C}{dt} = \tilde{f} N_A N_B - \tilde{b} N_C \tag{2.3}$$

which is an example of a chemical rate equation.

Chemical rate equations are usually written in terms of concentrations, which are measured in molar units (number of moles of a substance per litre). Let $[C]$ denote the molar concentration of C, then

$$[C] = \frac{N_C}{n_A V} \tag{2.4}$$

where $n_A \simeq 6.02 \times 10^{23}$ is Avogadro's number and V is the volume of the cell in litres. To convert Eq. 2.3 into an equation for the rate of change of the concentration of C, we must divide Eq. 2.3 by $n_A V$. This division gives

$$\frac{d[C]}{dt} = \tilde{f} n_A V [A][B] - \tilde{b}[C] \tag{2.5}$$

where $[A]$ is the concentration of A and $[B]$ is the concentration of B. If we define macroscopic reactions rates, for reactions involving concentrations, as

$$\begin{aligned} f &= \tilde{f} n_A V \\ b &= \tilde{b} \end{aligned} \tag{2.6}$$

then

$$\frac{d[C]}{dt} = f[A][B] - b[C]. \tag{2.7}$$

The units of the macroscopic rate f are $\mathrm{M}^{-1}\ \mathrm{s}^{-1}$. This rate of association should not change with volume because molecular species are now measured in concentrations. The rate of association of a pair of molecules, $\tilde{f}$, does, however, depend on volume and will decrease in larger volumes because it is more difficult for molecules to find each other. The volume-dependence in Eq. 2.6 cancels out the volume-dependence of $\tilde{f}$. In contrast, the units of the macroscopic rate b are unchanged (remaining s^{-1}) because b describes a dissociation reaction which always occurs at a rate independent of volume.

2.1.1 Example: dimerization

Many membrane receptors reversibly dimerize to form a receptor-receptor dimer and transcription factors often dimerize before binding to DNA, but the dimerization reaction is unusual. Let T denote a transcription factor and T_2 denote a dimer of two transcription factors. These species satisfy the reaction

$$T + T \underset{b}{\overset{f}{\rightleftharpoons}} T_2$$

The rate equations for this system are atypical because two molecules of T are removed by the f reaction and two molecules are released by the b reaction. Although the association reaction proceeds at the rate $f[T]^2$ and the dissociation reaction proceeds at the rate $b[T_2]$, we have

$$\frac{d[T]}{dt} = -2f[T]^2 + 2b[T_2] \tag{2.8}$$

because two T molecules are involved in both reactions. The dimer, T_2, obeys

$$\frac{d[T_2]}{dt} = f[T]^2 - b[T_2] \tag{2.9}$$

because only one molecule of dimer either forms or dissociates. Summing Eq. 2.8 and twice Eq. 2.9 gives

$$\frac{d[T]}{dt} + 2\frac{d[T_2]}{dt} = 0 \tag{2.10}$$

implying that

$$[T] + 2[T_2] = \text{constant} = [T]_0 + 2[T_2]_0 \tag{2.11}$$

where $[T]_0$ is the initial concentration of monomers and $[T_2]_0$ is the initial concentration of dimers. The dimerization reaction only changes the form of T molecules, either from monomers to dimers or vice versa, and does not lead to either the synthesis or destruction of T molecules. Consequently, the number of T molecules is conserved (and determined by the initial numbers of monomers and dimers). The conservation law, Eq. 2.11, reflects that a dimer contains twice as many T molecules as a monomer.

2.1.2 Diffusion-limited reactions

Association rates are expected to be less than $\simeq 10^9$ M^{-1} s^{-1}. All association reactions proceed by the two reactants first finding each other and then reacting. We can estimate the fastest rate at which such association reactions can possibly proceed by assuming that the reactants react immediately once together ($t_{\mathrm{reac}} = 0$ in Eq. 2.1). The upper bound on association reactions is then determined from the time taken for the two reactants to diffuse together (t_{diff}). Using the diffusion equation and assuming spherical reactants, this maximum rate is [1] (p. 314)

$$f_{\max} = 4\pi D a \tag{2.12}$$

where D is the sum of the diffusion constants of the reactants and a is the typical size of a reactant.

Remembering that D is measured in units of m^2 s^{-1}, note that $f_{\max}$ has units of volume per second and is the inverse of the time for a pair of reactants to diffuse together in a unit volume. We would like to convert these units to M^{-1} s^{-1} to be able to compare with standard association rates. We therefore multiply by Avogadro's number to convert to moles (similarly to Eq. 2.6) and by 10^3 to convert the volume units from m^3 to litres:

$$f \text{ (in M)} < f_{\max} \times n_a \times 10^3. \tag{2.13}$$

If D is 1000 $\mu\mathrm{m}^2$ s^{-1} (of order the diffusion constant of water [2] and around 150 times larger than the typical diffusion coefficients in the cytoplasm [3]) and a is 1 nm, then

$$\begin{aligned} f &< 4\pi \times 10^3 \times 10^{-12} \times 10^{-9} \times 6 \times 10^{23} \times 10^3 \\ &\simeq 7.5 \times 10^9 \,\mathrm{M}^{-1}\mathrm{s}^{-1}. \end{aligned}$$

2.1.3 The concentration of one molecule

A bacterium such as *Escherichia coli* has a volume of approximately 1 $\mu\mathrm{m}^3$ or 10^{-18} m^3 or 10^{-15} litres. The concentration of one molecule is then $1/n_A/10^{-15}$ M or of order 1 nM. The budding yeast *Saccharomyces cerevisiae* has a volume of approximately 60 $\mu\mathrm{m}^3$ or 60×10^{-15} litres. The concentration of one molecule is then of order 10 pM. A human fibroblast has a volume of approximately 10^4 $\mu\mathrm{m}^3$ and so the concentration of one molecule is of the order of 0.1 pM.

2.2 Modelling signal transduction I

To begin our model of a biochemical signalling pathway, consider a receptor, R, in the plasma membrane that enters an activated state R^* when bound by an extracellular signalling molecule,

S (Fig. 1). We can model this activation by a binary reaction:

$$[\mathrm{R}] + [\mathrm{S}] \underset{b}{\overset{f}{\rightleftharpoons}} [\mathrm{R}^*]$$

To allow the activated receptors to activate in turn a downstream signalling protein, A say, we include another binary reaction:

$$[\mathrm{R}^*] + [\mathrm{A}] \xrightarrow{k} [\mathrm{R}^*] + [\mathrm{A}^*]$$

Here $[R^*]$ appears on both sides of the chemical equation because R^* is not consumed by the reaction, but catalyzes the conversion of A to its activated form A^*.

The corresponding differential equations are

$$\begin{aligned}
\frac{d[S]}{dt} &= -f[R][S] + b[R^*] \\
\frac{d[R]}{dt} &= -f[R][S] + b[R^*] \\
\frac{d[R^*]}{dt} &= f[R][S] - b[R^*] \\
\frac{d[A]}{dt} &= -k[A][R^*] \\
\frac{d[A^*]}{dt} &= k[A][R^*]
\end{aligned}$$

but our main focus of interest is the production of A^* because A^* signals to the interior of the cell that molecules of S are present exterior to the cell.

We will therefore assume that the binding of S to R is at equilibrium so that

$$f[R][S] \simeq b[R^*]. \tag{2.14}$$

The number of receptor molecules is conserved because $d[R]/dt + d[R^*]/dt = 0$: receptors are neither created nor destroyed but only change state from inactivated to activated and vice versa. Writing R_0 for the total concentration of receptors so that

$$R_0 = [R] + [R^*], \tag{2.15}$$

then, using Eq. 2.14, we can show that

$$[R^*] \simeq \frac{[S]R_0}{\frac{b}{f} + [S]}. \tag{2.16}$$

The differential equation for $[A^*]$, the output of the signalling system, then becomes

$$\frac{d[A^*]}{dt} \simeq \frac{k[S]R_0}{\frac{b}{f} + [S]}[A] \tag{2.17}$$

or

$$\frac{d[A^*]}{dt} \simeq \frac{k[S]R_0}{\frac{b}{f} + [S]}(A_0 - [A^*]) \tag{2.18}$$

because the number of A molecules is conserved (with a total concentration of A_0) because A also only changes state.

Eq. 2.18 is our model of the signalling pathway. If either $[S] = 0$ or $f = 0$, no A^* is produced. If $[S] \gg b/f$, the rate of production of A^* saturates because all the receptors are bound by S. There is no reverse reaction that converts A^* back into A, and so all the A molecules become activated at steady-state: $[A^*] = A_0$.

2.3 Equilibrium and detailed balance

In the absence of any input of energy, chemical reactions reach an equilibrium where the number of molecules of each species stay constant. All time derivatives are then zero, and the system is said to be at a steady-state. Equilibrium is, however, a particular steady-state where detailed balance also holds. Detailed balance means that for each chemical reaction, the forward rate of the reaction must equal the backward rate of the reaction. When $[A]$, $[B]$, and $[C]$ all become constant,

$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = \frac{d[C]}{dt} = 0 \tag{2.19}$$

and the system is at steady-state. To be at equilibrium, we need detailed balance and so that

$$f[A][B] = b[C] \tag{2.20}$$

and the rate of association of A and B equals the rate of dissociation of C. For this system, there is one steady-state, which is equilibrium. For more complex systems, steady-state need not be equilibrium. For example, systems that contain irreversible reactions can reach steady-state but can never be at equilibrium because the backward rate of a irreversible reaction is zero and therefore cannot equal the forward rate: detailed balance does not hold.

The equilibrium dissociation constant is defined as $K_{\text{eq}} = b/f$, and the system at equilibrium then obeys

$$[A][B] = K_{eq}[C]. \tag{2.21}$$

The association and dissociation reactions do not create or destroy mass. The rate equations for $[A]$ and $[B]$ are

$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = -f[A][B] + b[C] \tag{2.22}$$

and, from Eq. 2.7, we can see that

$$\frac{d[A]}{dt} + \frac{d[C]}{dt} = 0 \tag{2.23}$$

implying

$$[A] + [C] = [A]_0 + [C]_0 \tag{2.24}$$

where $[A]_0$ and $[C]_0$ are the initial concentrations of A and C. This conservation law arises because each C molecules 'contains' an A molecule: a C molecule is an association of an A and B molecule. A similar conservation exists for B molecules: $[B] + [C] = [B]_0 + [C]_0$. Together with Eq. 2.21, these conservation laws define the equilibrium concentrations of $[A]$, $[B]$, and $[C]$.

2.3.1 Optional: Equilibrium and free energy

The equilibrium constant is determined by the change in free energy of a reaction. The free energy of a system is the energy available in the system to do work, i.e. to drive a process [2]. At equilibrium, the free energy is at a minimum. Consider the reaction

$$\mathrm{s_1X_1} + \cdots + \mathrm{s_kX_k} \rightleftharpoons \mathrm{s_{k+1}X_{k+1}} + \cdots + \mathrm{s_mX_m} \tag{2.25}$$

where k reactants react to from $m-k$ products, then

$$\frac{[X_{k+1}]^{s_{k+1}} \cdots [X_m]^{s_m}}{[X_1]^{s_1} \cdots [X_k]^{s_k}} = K_{\mathrm{eq}} \tag{2.26}$$

at equilibrium. The integers s_k are called the stoichiometric coefficients of the reaction. From statistical mechanics and assuming dilute solutions [2],

$$K_{\mathrm{eq}} = c_0^{s_{k+1}+\cdots+s_m-(s_1+\cdots s_k)}\, \mathrm{e}^{\frac{-\Delta G^{(0)}}{k_B T}} \tag{2.27}$$

where $\Delta G^{(0)}$ is the standard change in free energy caused by the occurrence of a single forward reaction, k_B is Boltzmann's constant, T is temperature, and c_0 is a reference concentration. The reference concentration is typically set to $c_0 = 1\mathrm{M}$.

We use the Gibbs free energy because intracellular chemical reactions typically occur at constant pressure and temperature. The Gibbs free energy is defined as $E+pV-TS$ where E is the system's energy, p is the system's pressure, V is the system's volume, and S is the system's entropy. Most biological reactions occur at constant pressure in solution with little change in volume when a reaction occurs. We therefore need not worry about the distinction between enthalpy, $E+pV$, and energy, E, because pV is almost constant, and need not discriminate between Helmholtz and Gibbs free energies [2].

For biological systems at constant temperature and pressure, the change in free energy can be written as [1]

$$\Delta G = \sum_j \mu_j \Delta N_j \tag{2.28}$$

and is determined solely by changes in numbers of molecules and by the chemical potentials, μ_j, of these molecules. The chemical potential for a reactant is the change in the free energy caused by removing a molecule from solution; the chemical potential for a product is the change in free energy caused by releasing a molecule into solution. If ΔG, the free energy change of the reaction, is negative, a chemical reaction will run forwards; if ΔG is positive, the chemical reaction will run backwards.

Mathematically, the chemical potential is defined as

$$\mu = k_B T \log(c/c_0) + \mu^{(0)} \tag{2.29}$$

with μ_0 being the standard chemical potential, which is a function of temperature and the properties of the chemical species being considered, such as its internal energy and mass [2].

For a system to reach equilibrium, it must minimize its free energy, which can occur through both decreasing the system's energy and increasing its entropy. At equilibrium, $\Delta G = 0$, and, from Eq. 2.28, the standard change in free energy of a reaction (the exponent in Eq. 2.27) is a sum of negative terms for each reactant and positive terms for each product with each term

being the product of the stoichiometric coefficient (the magnitude of ΔN_k is s_k) and the standard chemical potential for that chemical species.

Eq. 2.27 is useful because it is often easier to think in terms of changes of free energies rather than changes in equilibrium constants. For example, two transcription factors binding to DNA do so with an equilibrium constant of, say, K_{11}. If the transcription factors do not interact on the DNA, then the change in free energy of both transcription factors binding should correspond to the sum of the change in free energy of each transcription factor binding individually: $\Delta G_{11} = \Delta G_{10} + \Delta G_{01}$, with ΔG_{10} being the change in free energy of a transcription factor binding to one site and ΔG_{01} being the change in free energy of a transcription factor binding to the other site. Eq. 2.27 with $c_0 = 1\text{M}$ implies then that $K_{11} = K_{01}K_{10}$.

Example: Considering again the reaction

$$\text{A} + \text{B} \underset{b}{\overset{f}{\rightleftharpoons}} \text{C}$$

the change in free energy is

$$dG = \mu_A dN_A + \mu_B dN_B + \mu_C dN_C \tag{2.30}$$

and at equilibrium

$$\mu_A dN_A + \mu_B dN_B + \mu_C dN_C = 0 \tag{2.31}$$

because the free energy is at a minimum, and $dG = 0$. From the conservation laws,

$$N_A + N_C = \text{constant} \quad ; \quad N_B + N_C = \text{constant} \tag{2.32}$$

we have that $dN_A + dN_C = dN_B + dN_C = 0$. Therefore, Eq. 2.31 becomes

$$(-\mu_A - \mu_B + \mu_C) dN_C = 0 \tag{2.33}$$

or

$$-\mu_A - \mu_B + \mu_C = 0 \tag{2.34}$$

because $dN_A = dN_B = -dN_C$. Using the definition of μ, Eq. 2.29, we then have

$$-k_B T \log([A]/c_0) - \mu_A^{(0)} - k_B T \log([B]/c_0) - \mu_B^{(0)} + k_B T \log([C]/c_0) + \mu_C^{(0)} = 0 \tag{2.35}$$

Rearranging implies that

$$k_B T \log\left(\frac{[C]c_0}{[A][B]}\right) = \mu_A^{(0)} + \mu_B^{(0)} - \mu_C^{(0)} \tag{2.36}$$

or

$$\frac{[C]}{[A][B]} = c_0^{-1} \exp\left[-\frac{\mu_C^{(0)} - \mu_A^{(0)} - \mu_B^{(0)}}{k_B T}\right] \tag{2.37}$$

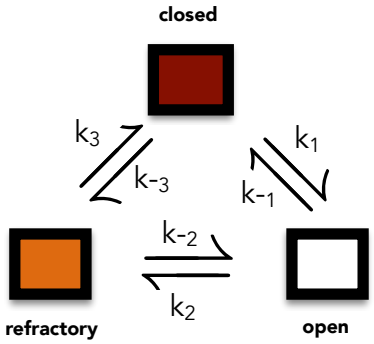
which is Eq. 2.27 for a reversible binary reaction.

2.4 The law of mass action

The law of mass action, developed by Guldberg and Waage in 1864, states that the rate of a reaction should depend on stoichiometry in the same way that equilibrium constants depend on stoichiometry. The stoichiometry of a reaction is defined as the relative numbers of reactants and products that are expended and created by the reaction (the s_i in Eq. 2.25). For example, for the association reaction $A + B \rightarrow C$, the stoichiometric coefficient of A is -1, of B is -1, and of C is 1 because one molecule of A combines with one molecule of B to form one molecule of C. Comparing Eq. 2.7 and Eq. 2.20, we can see that the dependence on stoichiometry is the same (the concentrations are raised to the same powers). Effectively, the law of mass action means that the rate of a reaction is proportional to the number of ways the reaction can occur, which is the logic we used to derive Eq. 2.7. Using the law of mass action, we ensure that the dynamics of our system are such that the system should reach a thermodynamically correct equilibrium.

2.5 Thermodynamic cycles

Ion channels often undergo thermodynamic cycles. They can switch from a closed to an open state, which allows ions to pass through the plasma membrane, and then frequently enter a refractory state. In the refractory state, the channel rarely opens but eventually transitions into the closed state, where switching to the open state is more probable. Schematically, we can write these reactions in a circle. The ion channel is said to undergo a thermodynamic cycle because the channel once open can go through the refractory and the closed state before opening again.



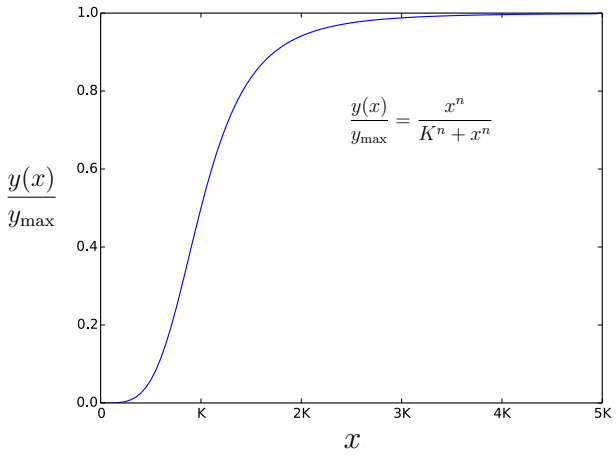
If we assume that each of these reactions is at equilibrium and so obeys detailed balance, then we can show that equilibrium imposes a constraint on the reaction rates:

$$k_1 k_2 k_3 = k_{-1} k_{-2} k_{-3}. \tag{2.38}$$

For a thermodynamic cycle that can reach equilibrium, the rate constants cannot be arbitrarily chosen and must obey Eq. 2.38, which implies that the probability of going round the cycle one way is equal to the probability of going round the cycle the other way. If the rate constants do not satisfy Eq. 2.38, the system is using energy to force the cycle to occur preferentially in one direction. A phenomenon that may not be intended by the modeller.

2.6 Ultrasensitivity and the Hill number

The response curve of system is the input-output relation and gives the level of output for all levels of input. Many response curves can be approximately described by a Hill function.



If the output, y, increases with increasing levels of input, x, the appropriate Hill function is

$$\frac{y(x)}{y_{\max}} = \frac{x^n}{K^n + x^n} \tag{2.39}$$

where n is called the Hill number, or occasionally the Hill coefficient, and K is the value of the input that causes the output to be half of its maximum value ($y_{\max}$). The parameter K is sometimes called the EC_{50} of the response or the half-maximal (50%) effective concentration.

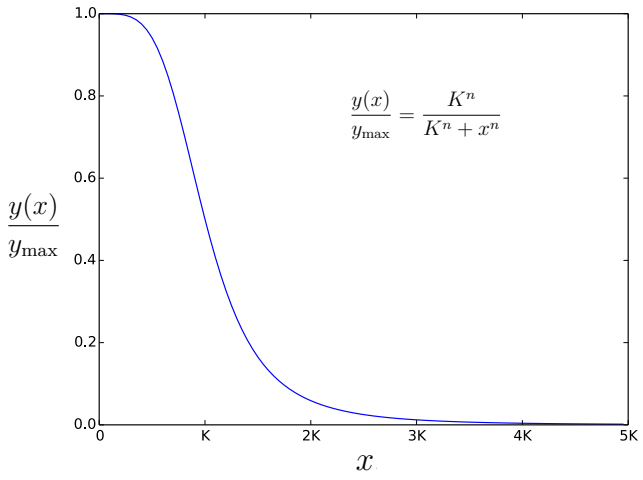
If the output decreases with increasing levels of input, then the approprate Hill function is

$$\frac{y(x)}{y_{\max}} = \frac{K^n}{K^n + x^n} \tag{2.40}$$

and K is now sometimes called the IC_{50} of the response or the half-maximal inhibitory concentration.

The Hill number is often used to characterize the ultrasensitivity or degree of cooperativity of the response. A response with a Hill number of 1 is said to be hyperbolic. The rate of a Michaelis-Menten enzymatic reaction as a function of the substrate concentration, Eq. 2.62, is a well-known example. If the Hill number is greater than 1, the response is ultrasensitive, and the response curve has a S- or sigmoidal shape. With Hill numbers above approximately 3, the response is switch-like or 'all-or-none' with little response for inputs below K and an almost maximal response for all inputs above K. This switch-like response is sometimes called a 'soft' switch because the underlying system is not bistable (Sec. 4).

For different biochemistry, there is different terminology; responses with a Hill number greater than 1 are often called ultrasensitive for systems involved in signal transduction; responses with a Hill number greater than 1 are often called cooperative for systems involved in gene regulation. A response with a Hill number below one is subsensitive.



2.7 Modelling signal transduction II

Considering Fig. 1, we can use a Hill function to immediately write an equivalent to Eq. 2.16:

$$[R^*] \simeq \frac{R_0[S]^n}{K^n + [S]^n} \tag{2.41}$$

where the Hill number n could be greater than 1 if, for example, multiple molecules of S have to bind to a receptor R to activate that receptor or if S only binds to R as a dimer. Eq. 2.18 then becomes:

$$\frac{d[A^*]}{dt} \simeq \frac{kR_0[S]^n}{K^n + [S]^n}(A_0 - [A^*]). \tag{2.42}$$

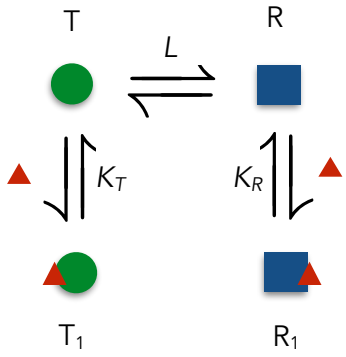
2.8 Allostery and the Monod-Wyman-Changeux model

Allostery is the modification of molecule's activity through binding of a regulatory molecule to a site on the molecule that is not the molecule's functional site. Allostery explains why a molecule that regulates an enzyme need not be of a similar structure to the substrate of an enzyme. That a regulatory molecule need not be similar to a substrate was a great revelation when discovered in the 1950s. Through conformational changes, binding sites on allosteric enzymes interact, and a molecule binding at a regulatory site cause an allosteric enzyme to change conformation at its active site and so alter enzymatic activity. Allostery is one way to generate ultrasensitive responses.

For example, a membrane receptor can become activated when bound by ligand. Biochemically, the receptor has two conformational states: an active and an inactive state. The active state can signal downstream; the inactivate state cannot. The binding of a ligand 'activates' the receptor by binding only to the active state and so stabilizing the receptor in this state. In the active state, the receptor may bind another signalling molecule on its cytoplasmic side and this molecule need have no structural relation to the ligand, which binds extracellularly to a different binding site on the receptor.

A celebrated model of allostery is the concerted model of Monod, Wyman, and Changeux [4]. In this model, an enzyme has two conformations – arbitrarily called a tense state (denoted T)

and a relaxed state (denoted R) – and spontaneously changes between these conformations. In the tense state, we will consider the enzyme to be 'on' and to have high activity; in the relaxed state, it is 'off' with low activity. In principle, any molecule that has a higher binding energy for the T state relative to the R state can activate the enzyme.



Let a regulatory molecule be X (shown as a red triangle), then in the absence of X, we can describe the spontaneous conformation changes as

$$\mathrm{T} \overset{L}{\rightleftharpoons} \mathrm{R}$$

where L is the equilibrium constant: $L = [R]/[T]$. The binding reactions are second-order:

$$\mathrm{X} + \mathrm{T} \overset{K_T}{\rightleftharpoons} \mathrm{T}_1$$

and

$$\mathrm{X} + \mathrm{R} \overset{K_R}{\rightleftharpoons} \mathrm{R}_1$$

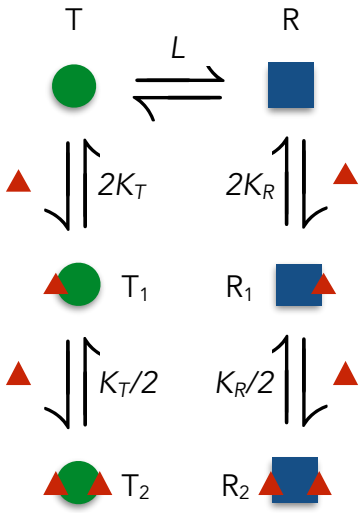
with K_T and K_R being association constants ($K_T[X][T] = [T_1]$ at equilibrium) and with $K_T > K_R$ so that X activates the enzyme (the T state is the 'on' state).

If there is only one binding site for X on the enzyme and assuming each reaction is at equilibrium, then the fraction of activated enzymes is

$$\begin{aligned} f_{\mathrm{on}} &= \frac{[T] + [T_1]}{[T] + [T_1] + [R] + [R_1]} \\ &= \frac{[T] + K_T[X][T]}{[T] + K_T[X][T] + L[T] + K_R[X]L[T]} \\ &= \frac{1 + K_T[X]}{1 + K_T[X] + L(1 + K_R[X])} \end{aligned}$$

which is a hyperbolic function increasing with $[X]$. The enzymatic activity increases with X because X biases the molecule to adopt the active conformation.

With more than one binding site for the regulatory molecule, X, the Monod, Wyman, and Changeux model assumes that all binding sites transition together, in a concerted manner, between the two conformational states [4]. Every binding site is therefore always in the same conformational state. Typically, these binding sites are on identical subunits of the molecule,



and we consider each subunit to have the same conformation. Other models of allostery relax this concerted assumption [5].

Allostery can give sharp, switch-like conditions as the concentration of the regulatory molecule changes. If the enzyme has two identical binding sites for X and f_T is the rate of binding one of those sites then the association reaction becomes

$$\mathrm{X} + \mathrm{T} \xrightarrow{2f_T} T_1$$

because there are now two choices of binding site for X. Denoting the rate of dissociation by b_T, the dissociation reaction remains

$$\mathrm{T}_1 \xrightarrow{b_T} \mathrm{X} + \mathrm{T}$$

because there is only one way that X can dissociate from T_1. The overall association constant is therefore $2f_T/b_T = 2K_T$.

We can understand this factor of two by considering explicitly the two binding sites for X on the enzyme. Let $T_{0,0}$ denote an enzyme in the tense state with no bound X molecules and $T_{1,0}$ denote a tense enzyme with an X bound to the first site and $T_{0,1}$ denote a tense enzyme with an X bound to the second site, then the reactions are

$$\mathrm{X} + \mathrm{T}_{0,0} \underset{b_T}{\overset{f_T}{\rightleftharpoons}} \mathrm{T}_{1,0}$$

and

$$\mathrm{X} + \mathrm{T}_{0,0} \underset{b_T}{\overset{f_T}{\rightleftharpoons}} \mathrm{T}_{0,1}$$

We can write down the differential equation for X, which will have four terms because of the four reactions:

$$\begin{aligned}\frac{d[X]}{dt} &= -f_T[X][T_{0,0}] - f_T[X][T_{0,0}] + b_T[T_{1,0}] + b_T[T_{0,1}] \\ &= -2f_T[X][T_{0,0}] + b_T[T_{1,0}] + b_T[T_{0,1}]\end{aligned}$$

If we define $[T_1] = [T_{1,0}] + [T_{0,1}]$ to be the concentration of the enzyme when one molecule of X is bound irrespective of which site on the enzyme has bound X, then Eq. 2.8 becomes

$$\frac{d[X]}{dt} = -2f_T[X][T_{0,0}] + b_T[T_1] \tag{2.43}$$

which is the differential equation that describes the reaction

$$\mathrm{X} + \mathrm{T} \underset{b_T}{\overset{2f_T}{\rightleftharpoons}} \mathrm{T}_1$$

with T written as $T_{0,0}$. The forward reaction rate increases by a factor 2 because we ignore the particular site to which X has bound and there are two possible sites on the enzyme where X can bind.

For the binding of a second X, we have

$$\mathrm{X} + \mathrm{T}_1 \xrightarrow{f_T} T_2$$

because there is only one binding site for X available on T_1. The dissociation of an X from T_2 can, however, occur in two ways depending on which X dissociates and whether either $T_{0,1}$ or $T_{1,0}$ forms, and so

$$\mathrm{T}_2 \xrightarrow{2b_T} \mathrm{X} + \mathrm{T}_1$$

The overall association constant is consequently $f_T/(2b_T) = K_T/2$.

Similar reactions hold for the binding of X to the R-state.

The fraction of activated enzyme can now be a sigmoidal function of the concentration of the regulatory molecule. Assuming equilibrium and so detailed balance for all reactions, the fraction of activated enzymes is

$$\begin{aligned}
f_{\mathrm{on}} &= \frac{[T] + [T_1] + [T_2]}{[T] + [T_1] + [T_2] + [R] + [R_1] + [R_2]} \\
&= \frac{[T] + 2K_T[X][T] + \frac{1}{2}K_T[X]2K_T[X][T]}{[T] + 2K_T[X][T] + \frac{1}{2}K_T[X]2K_T[X][T] + L[T] + 2K_R[X]L[T] + \frac{1}{2}K_R[X]2K_R[X]L[T]} \\
&= \frac{1 + 2K_T[X] + K_T^2[X]^2}{1 + 2K_T[X] + K_T^2[X]^2 + L(1 + 2K_R[X] + K_R^2[X]^2)} \\
&= \frac{(1 + K_T[X])^2}{(1 + K_T[X])^2 + L(1 + K_R[X])^2}
\end{aligned}$$

which is a sigmoidal function of $[X]$ with a maximum Hill number of 2.

For n binding sites,

$$f_{\mathrm{on}} = \frac{(1 + K_T[X])^n}{(1 + K_T[X])^n + L(1 + K_R[X])^n} \tag{2.44}$$

and the sharpness of the switch increases with a maximum Hill number of n (Fig. 2).

Limits of the Monod-Wyman-Changeux equation: We can build intuition about Eq. 2.44 by considering various limits.

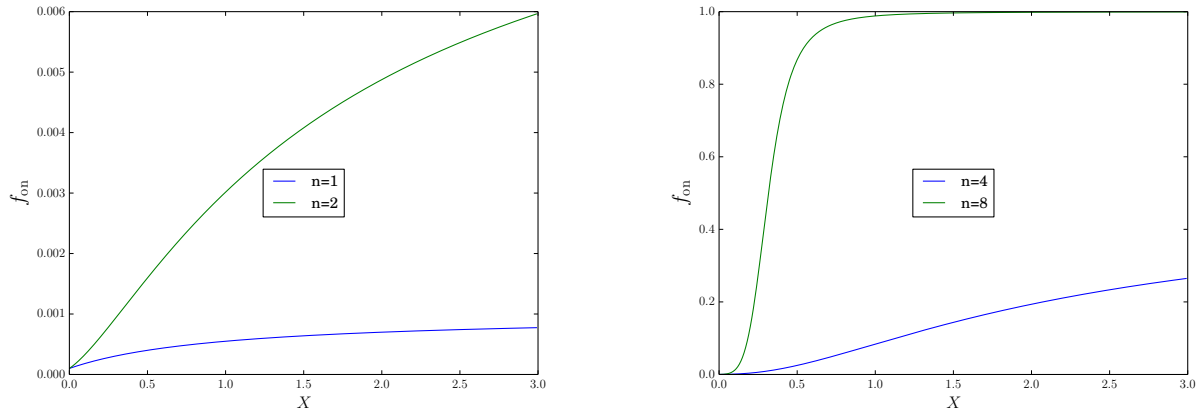


Figure 2: The response curve of an allosteric protein steepens with a higher number of binding sites for ligand. Here $L = 10^4$, $K_T = 10K_R$ and levels of ligand, X, are shown in units of K_R.

Eq. 2.44 always includes basal levels of activation. If there are no input molecules present

$$f_{\rm on}([X] = 0) = \frac{1}{1+L} \tag{2.45}$$

and there is basal activation unless $L \gg 1$ (remember that $[R] = L[T]$), which implies that molecules are only rarely in the active, tense state in the absence of input.

Full activation is only possible if the input strongly prefers binding to the tense state over binding to the relaxed state. If we add excess input molecules so that both $K_T[X] \gg 1$ and $K_R[X] \gg 1$, then Eq. 2.44 becomes

$$f_{\rm on}(K_R[X] \gg 1) \simeq \frac{K_T^n}{K_T^n + LK_R^n} \tag{2.46}$$

or, defining the bias in binding to be c so that $K_T = cK_R$,

$$f_{\rm on} \simeq \frac{c^n}{c^n + L} \tag{2.47}$$

and there is full activation if there is high bias: $c^n \gg L$.

Eq. 2.44 can become an activating Hill function for high bias and sufficient input. Writing Eq. 2.44 in terms of K_T and the bias $c = K_T/K_R$

$$f_{\rm on} = \frac{(1+K_T[X])^n}{(1+K_T[X])^n + L(1+K_T[X]/c)^n} \tag{2.48}$$

then if $c \gg K_T[X]$

$$f_{\rm on} \simeq \frac{(1+K_T[X])^n}{(1+K_T[X])^n + L}. \tag{2.49}$$

If too $K_T[X] \gg 1$, then

$$\begin{aligned} f_{\rm on} &\simeq \frac{(K_T[X])^n}{(K_T[X])^n + L} \\ &= \frac{[X]^n}{\frac{L}{K_T^n} + [X]^n} \end{aligned}$$

which is a Hill equation (Eq. 2.39) with a Hill number equal to n, the number of binding sites each allosteric molecule has for the input.

Eq. 2.44 can become an inhibiting Hill function for low bias and sufficient input. Writing Eq. 2.44 in terms of K_R and the bias $c = K_T/K_R$

$$f_{\text{on}} = \frac{(1 + cK_R[X])^n}{(1 + cK_R[X])^n + L(1 + K_R[X])^n} \tag{2.50}$$

then if $c \ll \frac{1}{K_R[X]}$

$$f_{\text{on}} \simeq \frac{1}{1 + L(1 + K_R[X])^n}. \tag{2.51}$$

If too $K_R[X] \gg 1$, then

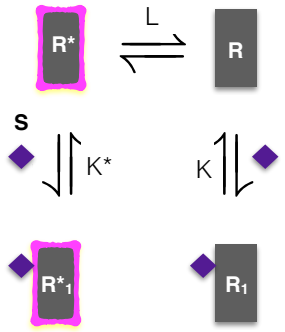
$$\begin{aligned} f_{\text{on}} &\simeq \frac{1}{1 + L(K_R[X])^n} \\ &= \frac{\frac{1}{LK_R^n}}{\frac{1}{LK_R^n} + [X]^n} \end{aligned}$$

which is also a Hill function (Eq. 2.40).

Allostery can therefore cause sharp switching of an enzyme between active and inactive states at a threshold concentration of the regulatory molecule. This cooperative behaviour arises because the first regulatory molecule is likely to bind to the conformational state of the enzyme that favours binding and so the enzyme will spend more time in this conformation making it easier for a second regulatory molecule to bind.

2.9 Modelling signal transduction III

We can use an allosteric model to describe activation of the receptors in Fig. 1. Consider



then the fraction of activated receptors is

$$f^* = \frac{1 + K^*[S]}{1 + K^*[S] + L(1 + K[S])} \tag{2.52}$$

from Eq. 2.8. The concentration of active receptors is $[R^*] = f^* R_0$, and so Eq. 2.42 becomes

$$\frac{d[A^*]}{dt} \simeq \frac{kR_0(1 + K^*[S])}{1 + K^*[S] + L(1 + K[S])}(A_0 - [A^*]). \tag{2.53}$$

When $[S] = 0$, then Eq. 2.53 simplifies

$$\frac{d[A^*]}{dt} \simeq \frac{kR_0}{1+L}(A_0 - [A^*]) \tag{2.54}$$

and there is a basal rate of activation even in the absence of ligand. This basal rate goes to zero as $L \gg 1$ because then receptors almost never spontaneously enter the activated state. If $K^*[S] \gg 1$ so that the vast majority of the activation of the receptors happens through the binding of S rather than spontaneously then

$$\frac{d[A^*]}{dt} \simeq \frac{kR_0K^*[S]}{L + (K^* + KL)[S]}(A_0 - [A^*]) \tag{2.55}$$

and we recover Eq. 2.18.

2.10 Enzyme kinetics

Almost all studies of enzymes start with the framework introduced by Michaelis and Menten, which although approximate is a general description. An enzymatic reaction is considered to occur in two steps: first, the enzyme binds the substrate to form an enzyme-substrate complex; second, catalysis occurs and this complex dissociates to form the product and release the enzyme:

$$\mathrm{E} + \mathrm{S} \underset{b}{\overset{f}{\rightleftharpoons}} \mathrm{C} \xrightarrow{k} \mathrm{P} + \mathrm{E}$$

For example, E may be a kinase in a signalling network that phosphorylates a substrate S to form P (phosphorylated S).

Using the law of mass action, the rate equations for this system are

$$\begin{aligned}
\frac{d[E]}{dt} &= -f[E][S] + (b+k)[C] \\
\frac{d[S]}{dt} &= -f[E][S] + b[C] \\
\frac{d[C]}{dt} &= f[E][S] - (b+k)[C] \\
\frac{d[P]}{dt} &= k[C].
\end{aligned}$$

Catalysis does not use up the enzyme, and we see that

$$\frac{d[E]}{dt} + \frac{d[C]}{dt} = 0 \tag{2.56}$$

so that

$$[E] + [C] = [E]_0 + [C]_0 = [E]_{\mathrm{tot}} \tag{2.57}$$

where the right-hand side is the total amount of enzyme initially present (denoted $[E]_{\mathrm{tot}}$). Similarly, substrate is converted into product and no new substrate is created, so that $[S] + [C] + [P]$ is a constant: the total amount of substrate is conserved in its various forms (either as free substrate, in complex with enzyme, or as product).

The Michaelis-Menten approximation typically relies on more substrate being present than enzyme, which is often true initially, so that almost all the enzyme is bound up in complex with the substrate most of the time. The concentration of the complex does not then change with time (although $[S]$ and $[P]$ do), at least while levels of S remain sufficiently high. We say that $[C]$ is at quasi-steady-state because $d[C]/dt \simeq 0$, but the system as a whole is not at steady-state ($d[S]/dt < 0$ and $d[P]/dt > 0$). If $d[C]/dt \simeq 0$, then

$$f[E][S] = (b+k)[C] \tag{2.58}$$

from Eqs. 2.10. Combining Eq. 2.58 with Eq. 2.57, we can show that

$$[C] \simeq \frac{[E]_{\text{tot}}[S]}{\frac{b+k}{f} + [S]} \tag{2.59}$$

and so

$$\frac{d[P]}{dt} \simeq \frac{k[E]_{\text{tot}}[S]}{\frac{b+k}{f} + [S]} \tag{2.60}$$

which depends only on the total amount of enzyme and the concentration of the substrate.

Defining

$$V_{\max} = k[E]_{\text{tot}} \quad ; \quad K_m = \tfrac{b+k}{f} \tag{2.61}$$

we have the Michaelis-Menten equation:

$$\frac{d[P]}{dt} \simeq \frac{V_{\max}[S]}{K_m + [S]} \tag{2.62}$$

for the initial rate of an enzymatic reaction. The maximum rate of the reaction is given by $V_{\max}$ and occurs for high concentrations of substrate. The concentration of substrate at which the reaction occurs at half this rate is given by the Michaelis constant, K_m.

The Michaelis-Menten equation is approximate, and more careful analysis shows that

$$\frac{[E]_{\text{tot}}}{[S]_0 + K_m} \ll 1 \tag{2.63}$$

is necessary for Eq. 2.62 to hold [6], where $[S]_0$ is the initial concentration of substrate. Eq. 2.62 is incorrect either when Eq. 2.63 is violated or for more complex enzymatic schemes such as those with multiple intermediate states.

2.11 Modelling signal transduction IV

Given our allosteric model of the activation of the receptors in Fig. 1 (Eq. 2.53), we can consider how the signal is propagated within the cell and model the dynamics of kinase B, which is activated by A^*. We will assume that this activation obeys Michaelis-Menten kinetics:

$$\mathrm{A}^* + \mathrm{B} \underset{b_B}{\overset{f_B}{\rightleftharpoons}} \mathrm{C_{AB}} \xrightarrow{k_B} \mathrm{B}^* + \mathrm{A}^*$$

The rate of change of $[B^*]$ then has a positive term

$$\frac{k_B[A^*][B]}{\frac{b_B+k_B}{f_B} + [B]} \tag{2.64}$$

from Eq. 2.60. If there is an enzyme that is constitutively active and de-activates B^*, such as a phosphatase if A^* is a kinase, then this enzyme too is likely to have Michaelis-Menten kinetics. Denoting the enzyme as P, we have

$$\mathrm{B}^* + \mathrm{P} \underset{b'_B}{\overset{f'_B}{\rightleftharpoons}} \mathrm{C_{PB}} \xrightarrow{k'_B} \mathrm{B} + \mathrm{P}$$

and so a negative term in the rate of change of $[B^*]$ of

$$-\frac{k'_B[P][B^*]}{\frac{b'_B+k'_B}{f'_B}+[B^*]}. \tag{2.65}$$

Hence

$$\frac{d[B^*]}{dt} \simeq \frac{k_B[A^*][B]}{\frac{b_B+k_B}{f_B}+[B]} - \frac{k'_B[P][B^*]}{\frac{b'_B+k'_B}{f'_B}+[B^*]} \tag{2.66}$$

or

$$\frac{d[B^*]}{dt} \simeq \frac{k_B[A^*](B_0-[B^*])}{\frac{b_B+k_B}{f_B}+B_0-[B^*]} - \frac{k'_B[P][B^*]}{\frac{b'_B+k'_B}{f'_B}+[B^*]} \tag{2.67}$$

because the total concentration of B is conserved and here equal to $B_0 = [B] + [B^*]$. Often the assumption that enzyme P works far from saturation is made so that $\frac{b'_B+k'_B}{f'_B} \gg [B^*]$. Eq. 2.67 then simplifies

$$\frac{d[B^*]}{dt} \simeq \frac{k_B[A^*](B_0-[B^*])}{\frac{b_B+k_B}{f_B}+B_0-[B^*]} - d_B[B^*] \tag{2.68}$$

where

$$d_B = \frac{f'_B k'_B[P]}{b'_B+k'_B}. \tag{2.69}$$

Including similar activation of molecule C by B^*, our final model of the cytoplasmic reactions of Fig. 1 is

$$\begin{aligned}
\frac{d[A^*]}{dt} &= \frac{k_A R_0(1+K^*[S])}{1+K^*[S]+L(1+K[S])}(A_0-[A^*]) - d_A[A^*] \\
\frac{d[B^*]}{dt} &= \frac{k_B[A^*](B_0-[B^*])}{\frac{b_B+k_B}{f_B}+B_0-[B^*]} - d_B[B^*] \\
\frac{d[C^*]}{dt} &= \frac{k_C[B^*](C_0-[C^*])}{\frac{b_C+k_C}{f_C}+C_0-[C^*]} - d_C[C^*]
\end{aligned}$$

assuming that deactivating enzymes (phosphatases) are present and are far from saturation.

2.12 Enzymatic cascades

Enzymatic cascades, where the first enzyme in the cascade activates the second and the second in turn activates the third and so on, have the potential to generate response curves that are ultrasensitive. A well understood example involves the MAP kinases involved in the maturation of oocytes in the frog *Xenopus laevis*. The hormone progesterone activates the MAP kinase kinase kinase Mos; Mos activates the MAP kinase kinase MEK1; and MEK1 activates the MAP kinase p42. Activation of p42 MAP kinase leads ultimately to maturation of the oocyte.

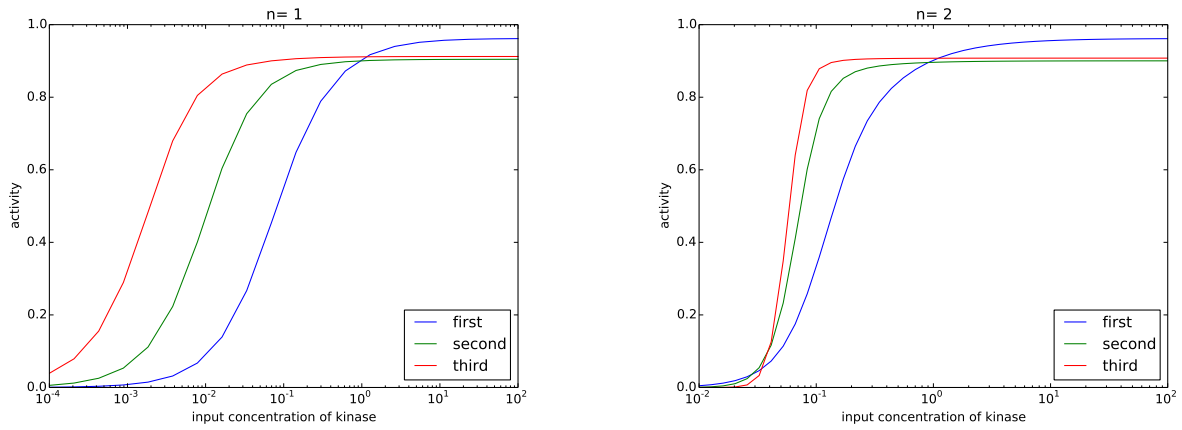


Figure 3: Enzymes lower in a cascade respond more sigmoidally than enzymes higher in the cascade if the Hill number for activation of each step, n, is greater than 1.

If each step of the cascade is ultrasensitive, then each subsequent step increases the ultrasensitivity of the response of the cascade's final enzyme. For example, if steady-state $[B^*]$ is a sigmoidal function of $[A^*]$ then

$$[B^*] = [B^*]_{\max} \cdot \frac{[A^*]^{n_B}}{K_B^{n_B} + [A^*]^{n_B}} \tag{2.70}$$

where n_B is the Hill number and K_B is the EC_{50} of the activation of B by A^*. Similarly, if steady-state $[C^*]$ is a sigmoidal function of $[B^*]$ then

$$[C^*] = [C^*]_{\max} \cdot \frac{[B^*]^{n_C}}{K_C^{n_C} + [B^*]^{n_C}} \tag{2.71}$$

where n_C is the Hill number and K_C is the EC_{50} of B^*. Inserting Eq. 2.70 into Eq. 2.71 gives

$$[C^*] = [C^*]_{\max} \cdot \frac{\left([B^*]_{\max} \frac{[A^*]^{n_B}}{K_B^{n_B}+[A^*]^{n_B}}\right)^{n_C}}{K^{n_C} + \left([B^*]_{\max} \frac{[A^*]^{n_B}}{K_B^{n_B}+[A^*]^{n_B}}\right)^{n_C}}. \tag{2.72}$$

If the concentration of A^* is smaller than its EC_{50} ($[A^*] \ll K_B$), then

$$[C^*] \simeq [C^*]_{\max} \cdot \frac{[A^*]^{n_B n_C}}{\frac{K_B^{n_B n_C} K_C^{n_C}}{[B^*]_{\max}^{n_C}} + [A^*]^{n_B n_C}} \tag{2.73}$$

and the maximum effective Hill number of the response of C^* to A^* is $n_B n_C$ — the product of the Hill numbers of each stage of the cascade. For example, if each element of the cascade has a Hill number of 2 then a cascade of three enzymes would have a maximum Hill number of $2^3 = 8$. In contrast, if each element of the cascade responds hyperbolically ($n = 1$) then the cascade will have a maximum Hill number of 1 regardless of the number of stages in the cascade (Fig. 3).

How could each element of the cascade have a Hill number greater than one? If a kinase needs to be phosphorylated only once by an upstream kinase then it is difficult to generate

ultrasensitivity without having to impose restrictions on the concentrations of the enzymes (through zero-order ultrasensitivity [7]). Many kinases, including many MAP kinases, require, however, two phosphorylations to become active. If the activating kinase acts distributively and dissociates from the downstream kinase after each phosphorylation (a processive kinase would bind its substrate and phosphorylate the substrate twice before dissociating), then intuitively activation of the downstream kinase 'sees' the concentration of the upstream kinase twice, once for each phosphorylation. We therefore might expect activation of the downstream kinase to be a sigmoidal function of the upstream kinase. Where tested, this expectation has been borne out [8].

2.13 Zero-order ultrasensitivity

A kinase and a phosphatase acting on the same substrate can generate a highly ultrasensitive response in the level of phosphorylated substrate as the ratio of the concentration of the two enzymes is varied [7]. A substrate that is continually phosphorylated and then dephosphorylated is sometimes said to take part in a 'futile' cycle because energy appears to be pointlessly consumed. Such cycles may, however, be used by the cell to generate ultrasensitive responses.

For example, consider a kinase and a phosphatase that bind identically to a substrate and either phosphorylate or dephosphorylate with the same rate. If there are initially equal amounts of both enzymes then half of the substrate is phosphorylated at steady-state. Let both enzymes be saturated – there is so much substrate compared to enzymes that both the kinase and the phosphatase work close to their maximum rate and no longer have a Michaelis-Menten dependence on the concentration of their substrate. If there is a small increase in the concentration of one of the enzymes, say the phosphatase, then the kinase is unable to resist the increase in phosphatase activity because the kinase is already working at its maximum rate. The extra phosphatases act as if they are unopposed, and there is a sharp switch in the phosphorylated state of the substrate with the substrate becoming mostly unphosphorylated. Similarly, a small increase in the concentration of the kinase away from the symmetric case leads to a switch to mostly phosphorylated substrate.

This ultrasensitive switch is referred to as 'zero-order' because both enzymes should be saturated and work at a constant, or zero-order, rate. If the enzymes are not saturated, then a small increase in the concentration of, say, the kinase can be opposed by the phosphatase: the activity of the phosphatase also increases because of the increase in concentration of phosphorylated substrate, following the Michaelis-Menten equation (Eq. 2.62). Zero-order ultrasensitive responses can switch sharply and can have Hill numbers greater than 10.

2.14 Summary

Models of biochemical systems are typically formulated using the law of mass action and so each chemical reaction proceeds at a rate proportional to the number of ways that the reaction can occur.

Without input of energy, all systems tend to equilibrium. Equilibrium is a special case of a steady-state condition, where each individual reaction is balanced by an opposing reaction. This condition of detailed balance means that no work can be extracted from the equilibrium state, and so all living cells can be at steady-state but not at equilibrium. Equilibrium in a

thermodynamic cycle imposes a condition on the rates of the reactions and they cannot all be freely chosen.

Several simplifying approximations that do not obey the law of mass action are often used for rates of reactions, but these approximations can be derived from system with dynamics that do obey the law of mass action. The Hill function describes a generic reaction rate that involves switch-like behaviour and does not satisfy mass action. The Michaelis-Menten rate of an enzymatic reaction has a Hill number of one, and the Monod-Wyman-Changeux model of allostery has a maximum Hill number determined by the number of subunits in the allosteric molecule. Ultrasensitive responses can have Hill numbers that give perfect switching (either all molecules 'off' or all molecules 'on'). Under certain conditions, cascades of switches have a Hill number that is the product of the Hill numbers for each individual stage.

3 Modelling gene expression

Gene expression is fundamental to much of biology and modelling gene expression is fundamental to much of systems biology. If we are interested in the average behaviour of a system, then modelling gene expression usually requires modelling the average state of occupancy of the promoter by transcription factors and RNA polymerase. We can use equations of chemical reactions to describe binding of proteins to the promoter and to describe transcription and translation.

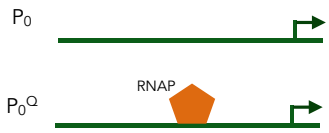
Binding of proteins to the DNA is assumed to occur faster than transcription, translation, and the degradation of both mRNAs and proteins so that each binding reaction is at equilibrium. We will derive expressions for the promoter occupancy from the assumption of equilibrium of DNA-binding reactions, but identical expressions can be written down directly using ideas from statistical mechanics [9].

3.1 Modelling constitutive expression

A constitutively expressed gene is one that is unregulated and synthesizes mRNA at a constant rate on average. The promoter therefore has two states: it can be either unbound or bound by RNA polymerase. If Q denotes RNA polymerase and P_0 is the unoccupied promoter then

$$\mathrm{P_0 + Q \rightleftharpoons P_0^Q}$$

is the binding reaction of RNA polymerase to the promoter. P_0^Q is the complex of the promoter bound by RNA polymerase:



At equilibrium,

$$P_0^Q = K_Q Q P_0 \tag{3.1}$$

where, for example, Q here represents the numbers of molecules of Q, which can be converted into a concentration $[Q]$ by dividing both sides of the equations by the volume of the cell. K_Q is an association constant.

The number of molecules of the promoter do not change with these reactions — the promoter only changes state — and, assuming n molecules of promoter, we can then write down a conservation law:

$$P_0 + P_0^Q = n \tag{3.2}$$

Using Eq. 3.1, this conservation implies that

$$P_0 + K_Q Q P_0 = n \tag{3.3}$$

and so that

$$\frac{P_0}{n} = \frac{1}{1 + K_Q Q} \tag{3.4}$$

which is the fraction of promoters that are not bound by RNA polymerase or, equivalently, the fraction of time that the promoter is free.

Transcription occurs only when RNA polymerase is bound and is modelled as

$$\frac{dM}{dt} = uP_0^Q - d_M M \tag{3.5}$$

where RNA polymerase initiates transcription with a rate u and mRNA, M, is degraded with first-order kinetics. The half-life of mRNA is $\log(2)/d_M$ because $\mathrm{e} = 2^{\frac{1}{\log 2}}$. Eqs. 3.1 and 3.4 imply that Eq. 3.5 can be written as

$$\frac{dM}{dt} = \frac{nuK_Q Q}{1 + K_Q Q} - d_M M. \tag{3.6}$$

We can see that the rate of transcription increases as the number of RNA polymerase molecules increase but saturates at a maximum rate of nu.

Translation is usually modelled as a first-order process:

$$\mathrm{M} \longrightarrow \mathrm{M} + \mathrm{P}$$

for mRNA, M, and protein, P. With first-order degradation of proteins, the equation for protein dynamics is then

$$\frac{dP}{dt} = vM - d_P P \tag{3.7}$$

with v being the rate of translation and d_P being the rate of degradation of proteins. The half-life of protein is $\log(2)/d_P$. In Eq. 3.7, M is a function of time and obeys Eq. 3.6.

Eqns. 3.6 and 3.7 together model constitutive gene expression.

3.2 Repression by a single repressor

The average rate of transcription is a Hill function of the concentration of repressor with a Hill number of one if the repressor binds to a single site on the DNA. Let P_0 denote the free promoter of a gene of interest and let P_1 denote the promoter when a repressor is bound. Then

$$\mathrm{P}_0 + \mathrm{R} \rightleftharpoons \mathrm{P}_1$$

for repressor, R. The binding of the repressor prevents RNA polymerase from binding to the promoter and so stops transcription. In the absence of repressor, RNA polymerase, denoted Q, can bind to the promoter

$$\mathrm{P}_0 + \mathrm{Q} \rightleftharpoons \mathrm{P}_0^{\mathrm{Q}}$$

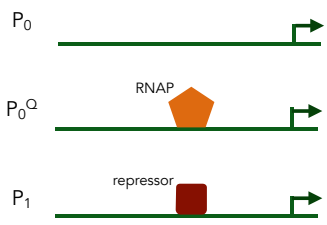
and initiate transcription with a rate u. If both these binding reactions are at equilibrium then

$$P_1 = K_R R P_0 \quad ; \quad P_0^Q = K_Q Q P_0 \tag{3.8}$$

where K_R and K_Q are both association constants and increase in magnitude if binding to the promoter becomes stronger.

The number of molecules of the promoter do not change with these reactions and, assuming n molecules of promoter, we can write:

$$P_0 + P_0^Q + P_1 = n \tag{3.9}$$



Using Eqs. 3.8, this conservation implies that

$$P_0 + K_Q Q P_0 + K_R R P_0 = n \tag{3.10}$$

and so that

$$\frac{P_0}{n} = \frac{1}{1 + K_Q Q + K_R R} \tag{3.11}$$

which is the fraction of time that the promoter is free and not bound by either the repressor or RNA polymerase (P_0 is the number of free promoters at equilibrium).

Transcription occurs only when RNA polymerase is bound

$$\frac{dM}{dt} = u P_0^Q - d_M M \tag{3.12}$$

Eqs. 3.8 and 3.11 imply that

$$\frac{P_0^Q}{n} = \frac{K_Q Q}{1 + K_Q Q + K_R R} \tag{3.13}$$

and so

$$\frac{dM}{dt} = \frac{n u K_Q Q}{1 + K_Q Q + K_R R} - d_M M \tag{3.14}$$

or

$$\frac{dM}{dt} = \frac{\left(\frac{n u K_Q Q}{1+K_Q Q}\right)}{1 + \left(\frac{K_R}{1+K_Q Q}\right) R} - d_M M \tag{3.15}$$

which has the form of a Hill function in the concentration of repressor if the number of free RNA polymerases is approximately constant. We can write

$$\frac{dM}{dt} = u_{\max} \left[\frac{1}{1 + \frac{R}{K_1}}\right] - d_M M \tag{3.16}$$

where the maximum rate of transcription is $u_{\max} = \frac{n u K_Q Q}{1+K_Q Q}$ and the half-maximal number of repressors is $K_1^{-1} = \frac{K_R}{1+K_Q Q}$. Note that both these quantities are functions of the numbers of free RNA polymerase, Q.

Translation can again be modelled as a first-order process:

$$\frac{dP}{dt} = v M - d_P P \tag{3.17}$$

where M satisfies Eq. 3.16.

3.3 Activation by a single activator

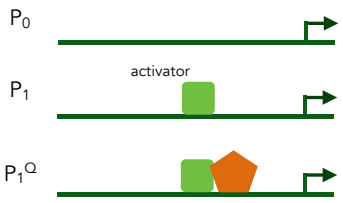
The average rate of transcription can also be a Hill function with a Hill number of one if transcription is controlled by the binding of a single activator. We can proceed as before and consider the binding of activator, A, to the free promoter

$$\mathrm{P_0 + A \rightleftharpoons P_1}$$

as well as the binding of RNA polymerase to the promoter when activator is already bound

$$\mathrm{P_1 + Q \rightleftharpoons P_1^Q}$$

and transcription only occurs from this state.



Assuming the number of promoters is conserved and that all reactions involving DNA binding are at equilibrium, then

$$P_1 = K_A A P_0 \quad ; \quad P_1^Q = K'_Q Q P_1, \tag{3.18}$$

where K_A and K'_Q are all association constants, and

$$P_0 + P_1 + P_1^Q = n \tag{3.19}$$

where n is the number of promoters.

Combining Eqs. 3.18 and 3.19 implies that

$$\frac{P_1^Q}{n} = \frac{K'_Q K_A A Q}{1 + K_A A + K'_Q K_A A Q} \tag{3.20}$$

for the fraction of time that the promoter is occupied by RNA polymerase. If u is the rate of transcription when both polymerase and activator are bound then mRNAs obey

$$\frac{dM}{dt} = \frac{u K'_Q Q K_A A}{1 + K_A A + K_A K'_Q Q A} n - d_M M \tag{3.21}$$

with first-order degradation of mRNAs. We can re-write the average rate of transcription as a function of only two parameters if Q is constant:

$$\begin{aligned} \frac{dM}{dt} &= \frac{(n u K'_Q Q) K_A A}{1 + (1 + K'_Q Q) K_A A} - d_M M \\ &= u_{\max} \left[\frac{\frac{A}{K_1}}{1 + \frac{A}{K_1}} \right] - d_M M \end{aligned}$$

with $u_{\max} = \frac{n u K'_Q Q}{1 + K'_Q Q}$ and $K_1^{-1} = (1 + K'_Q Q) K_A$, and the average transcriptional rate is a Hill function with a Hill number of one.

3.4 Activation by two activators

This approach can be extended to promoters that bind multiple transcription factors. For example, consider a promoter that has binding sites for two activators and can initiate transcription only when both binding sites are bound by activators. Denoting P_{00} as the free promoter, P_{10} and P_{01} as the promoter when one transcription factor is bound, and P_{11} as the promoter when two transcription factors are bound, then we have

$$\mathrm{P}_{00} + \mathrm{A} \rightleftharpoons \mathrm{P}_{10} \quad \text{and} \quad \mathrm{P}_{00} + \mathrm{A} \rightleftharpoons \mathrm{P}_{01}$$

and

$$\mathrm{P}_{01} + \mathrm{A} \rightleftharpoons \mathrm{P}_{11} \quad \text{and} \quad \mathrm{P}_{10} + \mathrm{A} \rightleftharpoons \mathrm{P}_{11}$$

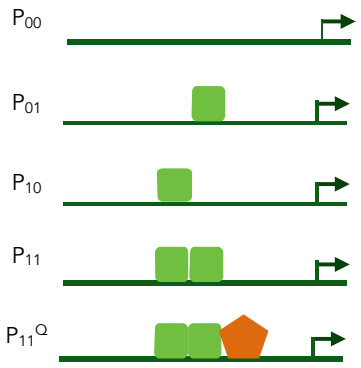
If these reactions are all at equilibrium, we can write

$$P_{10} = K_{10} A P_{00} \quad ; \quad P_{01} = K_{01} A P_{00} \tag{3.22}$$

and

$$P_{11} = \tilde{K}_{10} A P_{01} \quad ; \quad P_{11} = \tilde{K}_{01} A P_{10} \tag{3.23}$$

with K_{10}, K_{01}, $\tilde{K}_{10}$, and $\tilde{K}_{01}$ being association constants.



Eqns. 3.22 and 3.23 form a thermodynamic cycle and so a relationship exists between the equilibrium association constants

$$\tilde{K}_{10} K_{01} = \tilde{K}_{01} K_{10} \tag{3.24}$$

because at equilibrium there should be nothing unique about the route taken to form P_{11}, i.e. whether the activator binds initially to either the first or the second binding site. Finally, RNA polymerase can only bind to the promoter when both sites are bound by activators

$$\mathrm{P}_{11} + \mathrm{Q} \rightleftharpoons \mathrm{P}_{11}^{\mathrm{Q}}$$

and so

$$P_{11}^{Q} = K'_{Q} Q P_{11} \tag{3.25}$$

at equilibrium, with Q being the number of free polymerases.

The number of promoters is conserved

$$P_{00} + P_{01} + P_{10} + P_{11} + P_{11}^{Q} = n \tag{3.26}$$

which implies that

$$P_{00} + K_{10}AP_{00} + K_{01}AP_{00} + \tilde{K}_{10}K_{01}A^2P_{00} + \tilde{K}_{10}K_{01}K'_QQA^2P_{00} = n \tag{3.27}$$

and so

$$\frac{P_{11}^Q}{n} = \frac{K'_Q\tilde{K}_{10}K_{01}QA^2}{1 + K_{10}A + K_{01}A + \tilde{K}_{10}K_{01}A^2 + \tilde{K}_{10}K_{01}K'_QQA^2} \tag{3.28}$$

for the fraction of time the promoter is occupied by RNA polymerase.

Letting $\tilde{K}_{10} = K_iK_{10}$, with K_i greater than one and determined by the free energy of interaction between both activators when bound at the promoter, then the number of mRNAs obeys

$$\frac{dM}{dt} = \frac{unK'_QQK_iK_{10}K_{01}A^2}{1 + K_{10}A + K_{01}A + K_iK_{10}K_{01}A^2 + K_iK_{10}K_{01}K'_QQA^2} - d_MM \tag{3.29}$$

with u being the rate of transcription from promoter state P_{11}^Q. The average rate of transcription depends on three parameters if the number of free RNA polymerases is approximately constant

$$\frac{dM}{dt} = u_{\max}\left[\frac{\frac{A^2}{K_2^2}}{1 + \frac{A}{K_1} + \frac{A^2}{K_2^2}}\right] - d_MM \tag{3.30}$$

with $u_{\max} = \frac{unK'_QQ}{1+K'_QQ}$, $K_1^{-1} = K_{01} + K_{10}$, and $K_2^{-2} = K_iK_{10}K_{01}(1 + K'_QQ)$, and has a maximum Hill number of 2.

3.4.1 Multiple transcriptionally active states

We can extend this model by allowing RNAP to bind to the promoter in the absence of the activators too:

$$\mathrm{P_0 + Q} \rightleftharpoons \mathrm{P_Q}$$

with $P_Q = K_QQP_0$. If u_ℓ is the rate of transcription from this state (such unregulated transcription is sometimes called leakage), then Eq. 3.29 becomes

$$\frac{dM}{dt} = n\frac{u_\ell K_QQ + uK'_QQK_iK_{10}K_{01}A^2}{1 + K_QQ + K_{10}A + K_{01}A + K_iK_{10}K_{01}A^2 + K_iK_{10}K_{01}K'_QQA^2} - d_MM \tag{3.31}$$

with a new K_QQ term appearing in the numerator and the denominator because we are considering an additional state of the promoter that is transcriptionally active: the P_Q state. If Q is constant, we can simplify to write

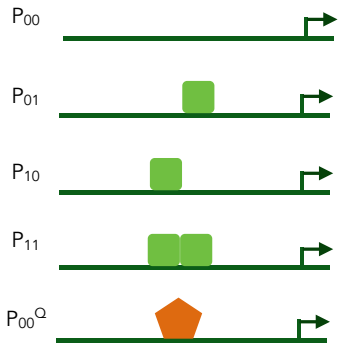
$$\frac{dM}{dt} = \frac{u_{\text{basal}}}{1 + \frac{A}{K_1} + \frac{A^2}{K_2^2}} + \frac{u_{\max} \times \frac{A^2}{K_2^2}}{1 + \frac{A}{K_1} + \frac{A^2}{K_2^2}} - d_MM \tag{3.32}$$

but now with $K_1^{-1} = \frac{K_{01}+K_{10}}{1+K_QQ}$ and $K_2^{-2} = K_iK_{10}K_{01}\frac{1+K'_QQ}{1+K_QQ}$ and with a basal rate of transcription of $u_{\text{basal}} = \frac{u_\ell nK_QQ}{1+K_QQ}$. As before, $u_{\max} = \frac{unK'_QQ}{1+K'_QQ}$. For the activators to be efficient, RNAP should prefer to bind to the promoter when the promoter is bound by the two activators ($K'_Q > K_Q$) and that the rate of transcription should be highest from this promoter state ($u > u_\ell$).

3.5 General regulation

There is a general pattern in the expressions for the average rate of transcription and this pattern is expected from statistical mechanics [9]. The denominator of the average rate of transcription is a sum of terms with each term representing a possible state of the promoter: the free state of the promoter is represented by the number 1 and a bound state is represented by the product of the association constants for each binding event times the number of ways those binding events can occur. The numerator of the average rate of transcription is a sum of similar terms but only those terms that represent states of the promoter from which transcription can occur. Each of these terms is multiplied by the rate of transcription from that state, and their sum is multiplied by the number of promoters.

For example, consider a promoter with two binding sites for repressors where the binding of a repressor to either site prevents the binding of RNA polymerase, then the promoter has five states: free, bound by polymerase, one site bound by a repressor, the other site bound by a repressor, and both sites bound by a repressor. The denominator of the average rate of



transcription is

$$1 + K_Q Q + K_{01} R + K_{10} R + K_{11} R^2 \tag{3.33}$$

and the numerator is

$$nu \times K_Q Q \tag{3.34}$$

The association constant K_{11} for two repressors binding simultaneously to the promoter should be determined by the change in free energy of one repressor binding, the change in free energy of another binding, and any free energy of interaction between the repressors once they are bound: $K_{11} = K_{01} K_{10} K_i$. Therefore the number of mRNAs satisfies the rate equation:

$$\frac{dM}{dt} = nu \frac{K_Q Q}{1 + K_Q Q + K_{01} R + K_{10} R + K_i K_{10} K_{01} R^2} - d_M M \tag{3.35}$$

which we can write as

$$\frac{dM}{dt} = \frac{nu K_Q Q}{1 + K_Q Q} \left[\frac{1}{1 + \frac{K_{01} + K_{10}}{1 + K_Q Q} R + \frac{K_i K_{10} K_{01}}{1 + K_Q Q} R^2} \right] - d_M M \tag{3.36}$$

and has a maximum Hill number of two.

3.6 Modelling signal transduction V

We can now add to the model of Sec. 2.11 , the expression of the reporter gene in Fig. 1, which is expressed through the action of C^*.

The nuclear entry and exit of C^* can be written as a chemical reaction:

$$\mathrm{C}^* \underset{b_n}{\overset{f_n}{\rightleftharpoons}} \mathrm{C}_\mathrm{n}^*$$

which, if at equilibrium, implies that

$$[C_n^*] = \frac{f_n}{b_n}[C^*]. \tag{3.37}$$

Making the simplest assumption that C_n^* is an activator that binds to a single binding site on the reporter gene, G, then the mRNA of G, m_G, satisfies (following Eq. 3.3)

$$\frac{d[m_G]}{dt} = u_G \frac{\frac{[C_n^*]}{K_{C^*}}}{1 + \frac{[C_n^*]}{K_{C^*}}} - d_m[m_G] \tag{3.38}$$

with a maximum transcription rate of u_G and a degradation rate d_m of the mRNA. The protein G, itself, obeys (following Eq. 3.17)

$$\frac{d[G]}{dt} = v[m_G] - d_G[G] \tag{3.39}$$

for translation rate v and degradation rate d_G of protein G.

Eqs. 2.11 with Eqs. 3.38 and 3.39 are the complete model of the pathway of Fig. 1 from the input S to the output G.

4 Positive feedback and bistability

Positive feedback, where an increase in the output of a system causes the output of the system to increase further, can generate a bistable response. For certain parameter values, a bistable system has two stable steady-states. If the system starts from one set of initial conditions (the initial values of all the concentrations) and evolves with time, it will always eventually reach one particular steady-state set of concentrations; if the same system starts from a different set of initial conditions, it will always eventually reach the other steady-state set of concentrations. Each steady-state has its own basin of attraction defined as all initial concentrations that evolve to that steady-state, and each set of initial concentrations must lie in one of the two basins of attraction. Intuitively, if the level of output does not get sufficiently high then the system tends to one steady-state; if the output gets high enough for the positive feedback to 'run away' and generate yet more output, then the system tends to the other steady-state.

4.1 MAP kinase cascades: a one dimensional example

Understanding how positive feedback generates multiple steady-states is best understood graphically. Consider the MAP kinase cascade in frog oocytes: activation of the last kinase of the cascade (indirectly through adding the hormone progesterone) causes new synthesis of the MAP kinase kinase kinase, Mos. There is thus positive feedback on activation of the entire cascade: increased activation of Mos increases activation of MAP kinase, which in turn increases activation of Mos by causing the synthesis of more Mos molecules.

Following Ferrell *et al.* [10], we consider three processes that control levels of Mos. First, there is a basal rate of synthesis that depends on progesterone:

$$\text{basal synthesis} = k_b[p] \tag{4.1}$$

where k_b is the basal rate and $[p]$ is the concentration of progesterone. Second, positive feedback occurs because synthesis of Mos is proportional to the concentration of activated MAP kinase. If we assume that the concentration of MAP kinase is a Hill function of the concentration of Mos, then this term is:

$$\text{positive feedback} = f\frac{[\text{Mos}]^n}{K^n + [\text{Mos}]^n} \tag{4.2}$$

where f measures the strength of the feedback. Finally, Mos is degraded intracellularly, which we model as a first order process:

$$\text{degradation} = -[\text{Mos}] \tag{4.3}$$

measuring units of time in units of the lifetime of Mos.

Consequently, the rate of change of the concentration of Mos is

$$\frac{d[\text{Mos}]}{dt} = k_b[p] + f\frac{[\text{Mos}]^n}{K^n + [\text{Mos}]^n} - [\text{Mos}] \tag{4.4}$$

and typical parameter values are $K = 20$ nM, $n = 5$, $k_b = 0.2$, and $f = 40$ [10].

At steady-state, the rate of synthesis of Mos equals the rate of degradation of Mos. Therefore to find steady-state values, we can plot the total synthesis rate as a function of the concentration of Mos and the total degradation rate as a function of Mos with any intersections between these

two curves determining a steady-state concentration of Mos (Fig. 4A). Notice that if the rate of synthesis of Mos was not ultrasensitive but hyperbolic, then the system would have only one stable steady-state and no switch-like behaviour (Fig. 4B).

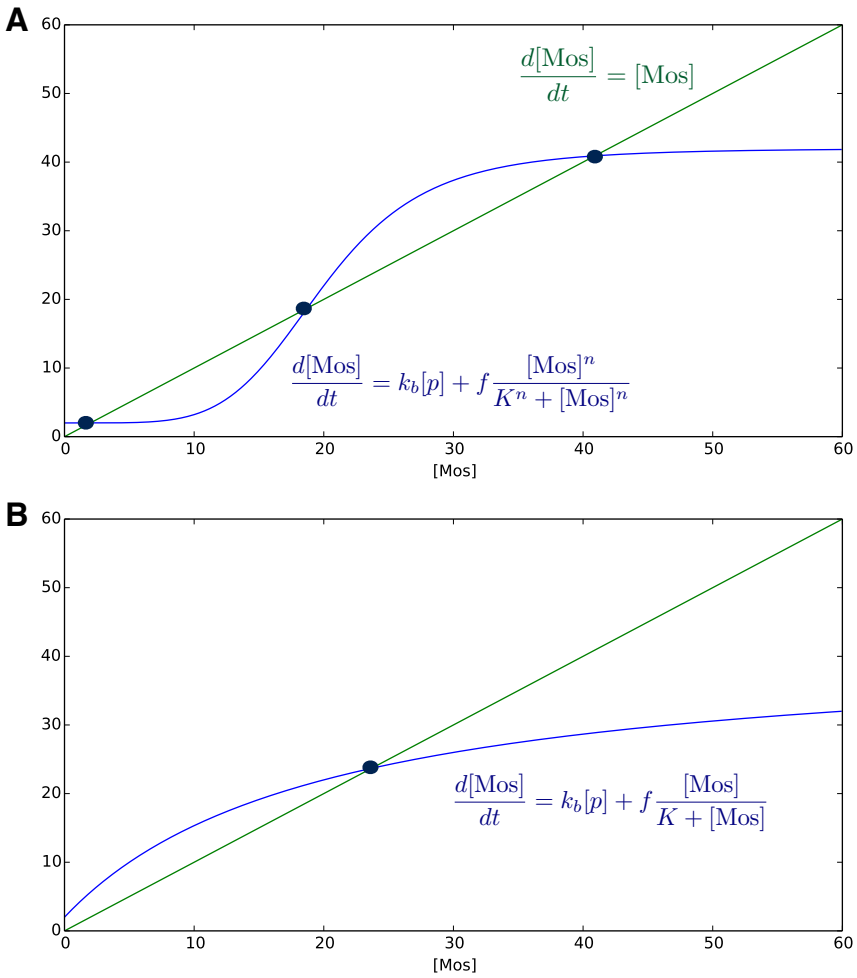


Figure 4: Steady-state solutions of Eq. 4.4 can be found using a graphical construction: the intersection of a curve describing the production rate of [Mos] as a function of [Mos] with a curve describing the degradation rate of [Mos] as a function of [Mos] gives the steady-state concentration. **A:** With a sigmoidal production rate, three steady-states exist of which two are stable. **B:** With a hyperbolic production rate there is only one steady-state.

Depending on the initial conditions, the system will tend to one of the two steady-states. It will avoid the unstable steady-state. Even if the system is initiated at the concentrations of the unstable steady-state, any perturbation, no matter how small, will cause the system to tend to one of the two steady-states (Fig. 5).

Figure 5: The phase portrait when $[p] = 20$ nM (see Fig. 6). If the initial [Mos] is above the value at the unstable steady-state, then [Mos] tends to the upper stable steady-state. If the initial [Mos] is below the value at the unstable steady-state, then [Mos] tends to the lower stable steady-state.

A bifurcation is a qualitative change in the dynamics of a system [11]. As we change the concentration of pheromone from, for example, low to high values, the number of steady-state concentrations of Mos changes from three to one (Fig. 6). This change in pheromone qualitatively changes the system's dynamics, and a bifurcation has occurred. When the system has one steady-state then this steady-state is stable (for example, when $[p] = 60$ nM), and the evolution of the system from any initial condition will ultimately lead to that steady-state. When the system has three steady-states (for example, when $[p] = 20$ nM), two steady-states are stable and the steady-state between these two steady-states is unstable.

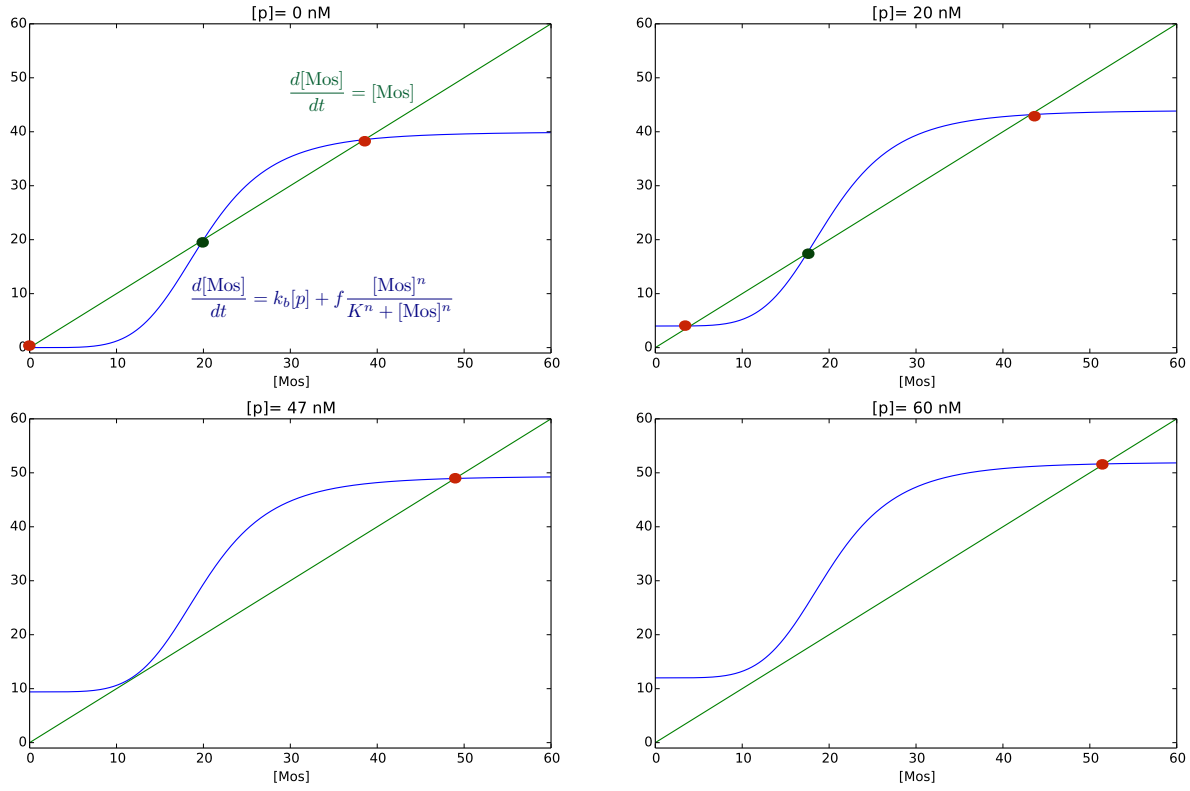


Figure 6: The number of stable steady-states (denoted in red) changes as the concentration of pheromone, which sets the intercept with the y-axis, changes.

As the concentration of pheromone is increased from zero, one stable and the unstable steady-state approach each other. At the bifurcation point, they annihilate each other and both disap-

pear (at $[p] \simeq 47$ nM). The system as a whole therefore is left with only one steady-state. This disappearance of a stable and an unstable steady-state is called a saddle-node bifurcation [11]. Such a bifurcation can also create a stable and an unstable node if, for example, pheromone is now decreased.

4.2 Bifurcation diagrams and hysteresis

A bifurcation diagram shows qualitative changes in the long-term behaviour of the output of a system as a function of a parameter in the system. For the MAPK system, we can plot the steady-state values of protein as a function of the progesterone concentration $[p]$ (Fig. 7). For low $[p]$, there are two stable steady-states for Mos (Fig. 6); for high $[p]$ there is one high steady-state (Fig. 6). Usually, stable steady-states are marked on bifurcation diagrams with solid lines and unstable steady-states are marked with dashed lines. The signalling system can therefore act as a switch with the steady-state level of Mos jumping from a low to a high value as the bifurcation parameter, here $[p]$, changes.

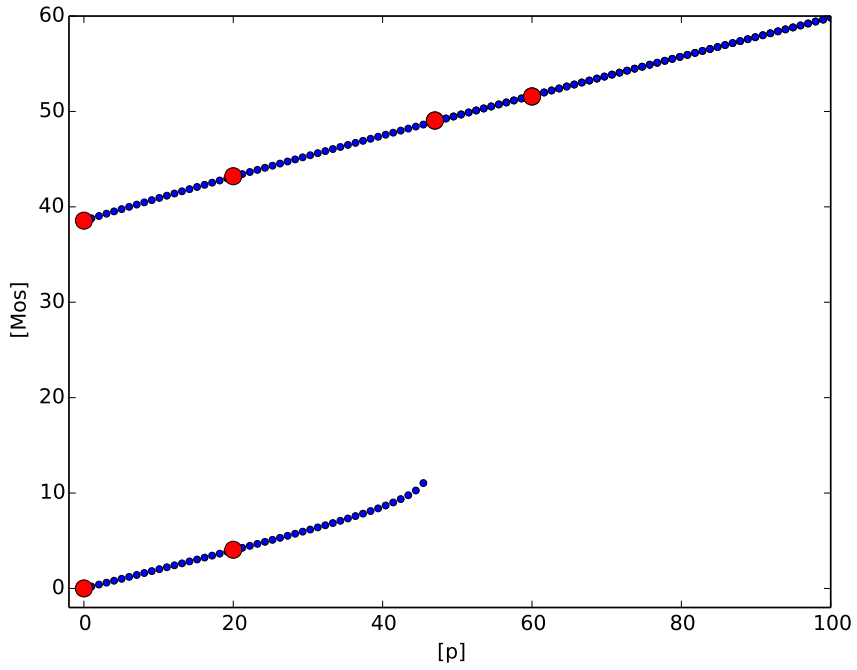


Figure 7: A bifurcation diagram showing the stable steady-state concentrations of Mos as a function of the concentration of pheromone. A saddle-node bifurcation occurs at $[p] \simeq 47$ nM. The stable steady-states in Fig. 6 are shown in red.

Bistable systems typically show history-dependent, or hysteretic, behaviour. As $[p]$ is increased from low to high values, the steady-state level of [Mos] jumps from a low to a high value at a particular threshold value of $[p]$ (when the system goes through a saddle-node bifurcation). Decreasing $[p]$ will, in this example, cause no jump back to the low state of Mos, and the system has a permanent memory, always remembering the exposure to the high progesterone concentration.

Next we will consider a system that has the potential for not one but two saddle-node bifurcations.

4.3 A genetic switch: a two dimensional example of a saddle-node bifurcation

In systems with many chemical species, bistability and saddle-node bifurcations occur analogously to the one dimensional case. For example, consider a protein that activates its own expression [11]. Writing M for mRNA and P for protein, this two dimensional system can be described by

$$\frac{dM}{dt} = v + \frac{uP^n}{K^n + P^n} - d_M M \quad ; \quad \frac{dP}{dt} = M - d_P P \tag{4.5}$$

with a Hill function describing the activation of transcription by P. Here d_M is the rate of degradation of mRNA; d_P is the rate of degradation of protein; u is the maximum rate of transcription induced by P; and v is a basal rate of transcription. The system has positive feedback because high levels of protein cause higher rates of transcription and so levels of protein that become higher still.

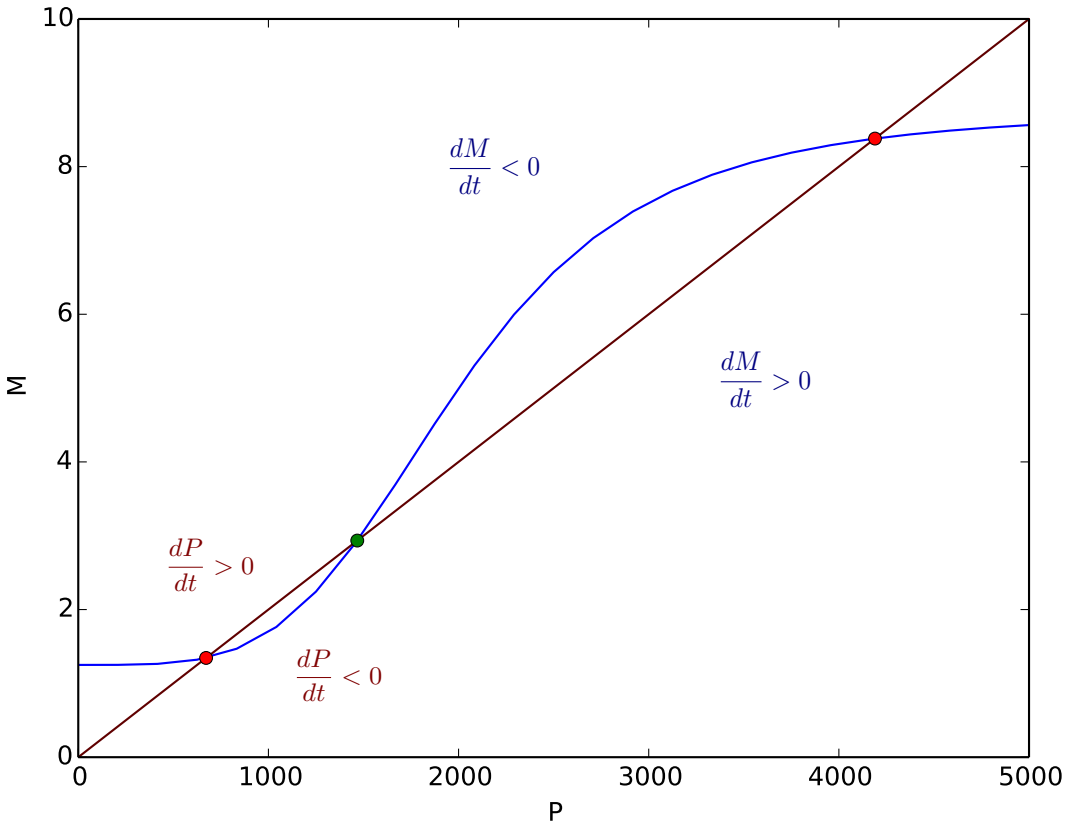


Figure 8: The intersection of the nullclines show the steady-states of the model of the genetic switch. Stable steady-states are shown in red; unstable steady states are shown in green. Here $n = 4$, $v = 0.01$ s^{-1}, $d_M = 0.008$ s^{-1}, $d_P = 0.002$ s^{-1}, $K = 2000$, and $u = 0.06$ s^{-1}.

In two dimensions, graphical approaches are commonly used to analyse bistable systems. At

steady-state, both dM/dt and dP/dt are zero. To find the possible steady-states of the system, we plot the nullclines, defined as the curves where either dM/dt or dP/dt are zero [11]. These curves are

$$M = \frac{1}{d_M}\left(v + \frac{uP^n}{K^n + P^n}\right) \quad ; \quad M = d_P P \tag{4.6}$$

from Eq. 4.5, and we plot both curves in the same P-M plane (Fig. 8). The steady-states are the points where the nullclines intercept: at these points, dM/dt and dP/dt must both zero.

The genetic switch shown in Fig. 8 has three steady-states. The middle steady-state is unstable; the other two steady-states are stable. The stable states are called stable nodes because they are attracting: a system starting near a stable node will move over time towards the node. The unstable steady-state is called a saddle point: a system near the saddle point is either immediately repelled from or initially is attracted towards and is then repelled from the saddle point. An unstable node in contrast repels all systems that are initialized sufficiently near it.

A phase portrait shows graphically the dynamics of the system. From Eqs. 4.5, dM/dt is negative above the $dM/dt = 0$ nullcline and so the dynamics there decreases M; dM/dt is positive below the $dM/dt = 0$ nullcline and so the dynamics in that region increases M (Fig. 8). Similarly, dP/dt is positive above the $dP/dt = 0$ nullcline and the dynamics there increases P, and dP/dt is negative below the $dP/dt = 0$ nullcline and the dynamics there decreases P. Using arrows to indicate the local direction of the dynamics, we can find the phase portrait (Fig. 9).

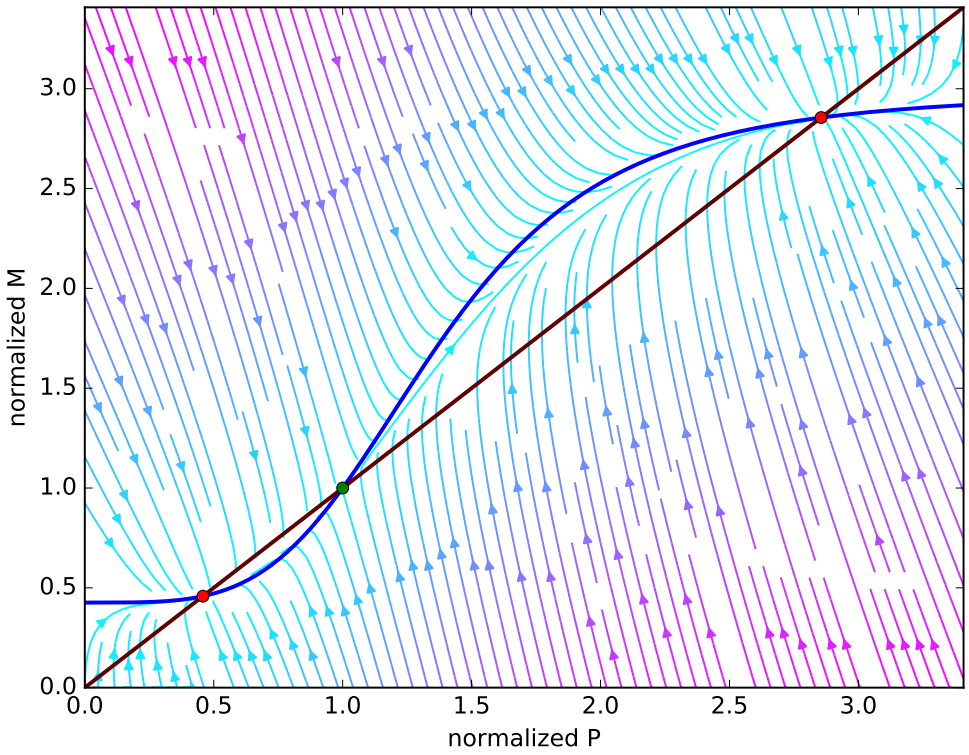


Figure 9: The phase portrait for the genetic switch shows two stable steady-states (in red) separated by an unstable steady-state (in green). Protein and mRNA are shown relative to their levels at the unstable steady-state.

As we change the degradation rate of protein, d_P, the system undergoes two saddle-node bifurcations. The number of intersections of the nullclines changes from one to three to one (Fig. 10). As d_P is changed, a stable steady-state, the node, and an unstable steady-state, the saddle, can either approach and annihilate each other and thus remove bistability or can be simultaneously created and this generate bistability. When the rescaled $d_P \simeq 0.8$, a saddle and a node are created if d_P is increasing and self-annihilate if d_P is decreasing. Similarly, when the rescaled $d_P \simeq 1.3$, a node and a saddle point self-annihilate if d_P is increasing and are created if d_P is decreasing (Fig. 10). Saddle-node bifurcations can create and destroy bistability in all dimensions.

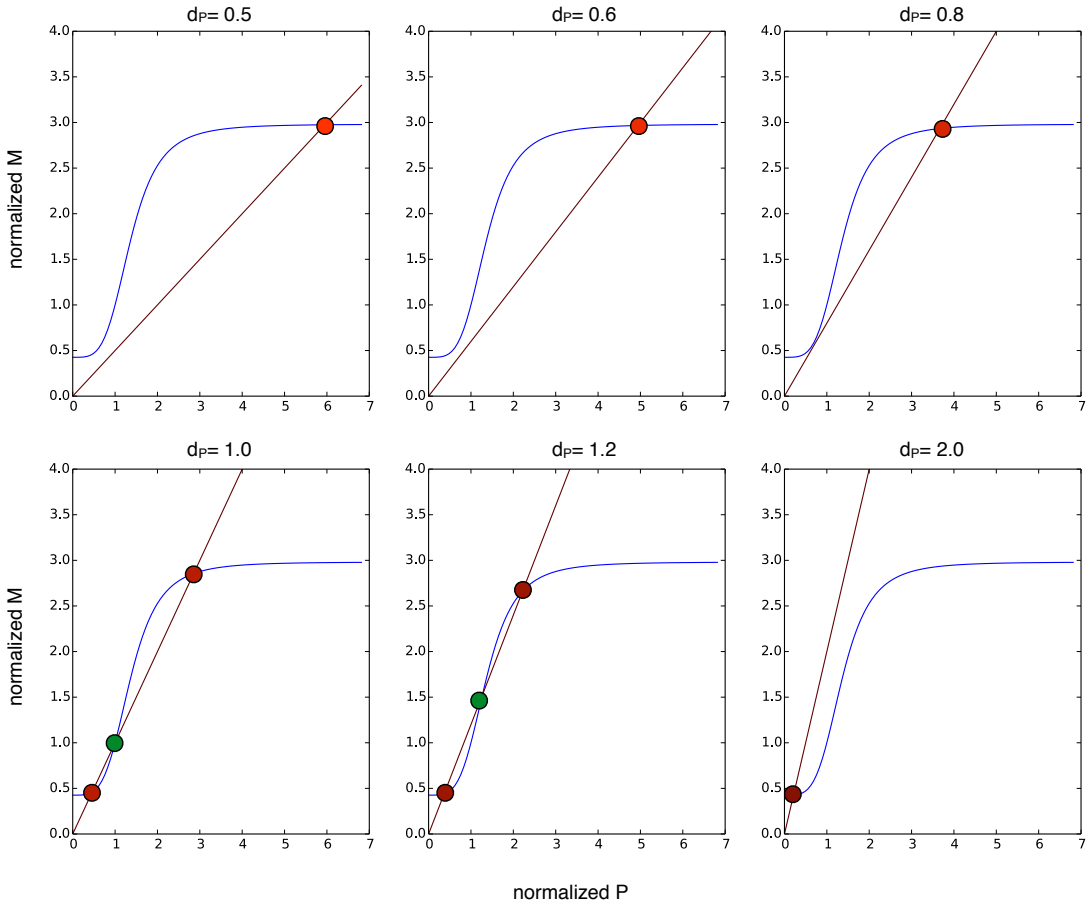


Figure 10: The system undergoes two saddle-node bifurcations as d_P is changed: one at the rescaled $d_P \simeq 0.8$ and the other at $d_P \simeq 1.3$ (not shown). Protein and mRNA are given relative to their levels at the unstable steady-state when $d_P = 0.002$ s^{-1}, and d_P is measured relative to 0.002 s^{-1}.

The system exhibits hysteresis, or history-dependent behaviour. As d_P is increased from low values, the system jumps from a high value of P to a low value at the bifurcation when the rescaled $d_P \simeq 1.3$ (Fig. 11). As d_P is decreased from high values, the system jumps from a low value of P to a high value at the bifurcation when the rescaled $d_P \simeq 0.8$ (Fig. 11). The value of the threshold when the jump occurs depends on the history of the change in d_P.

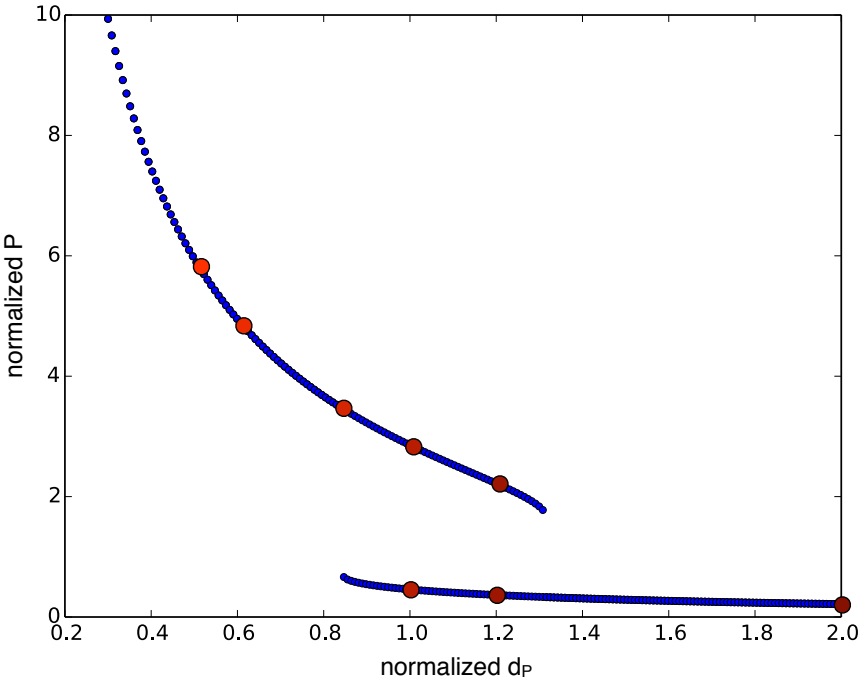


Figure 11: The bifurcation diagram for the genetic switch. The stable-steady states shown in Fig. 10 are shown in various shades of red. Protein and mRNA are shown relative to their levels at the unstable steady-state when $d_P = 0.002$ s^{-1}, and d_P is measured relative to 0.002 s^{-1}.

This behaviour is general. A system with positive feedback can exhibit hysteresis (history-dependent behaviour) because the value of the bifurcation parameter at which the system jumps is history-dependent: if the system was previously in the high state, then the threshold value is different from the threshold value of the jump if the system was previously in the low state. Observing hysteresis experimentally is usually considered sufficient proof that a system is bistable; observing bimodality in a response is consistent with bistability but not sufficient to prove bistability.

5 Negative feedback and oscillations

A limit cycle is an isolated and closed trajectory in phase space [11]. Remember that phase space is the space where the concentration of each chemical species in a network is plotted along each axis. An isolated trajectory means that neighbouring trajectories either spiral in towards the closed trajectory or spiral away from the closed trajectory. Once on a stable limit cycle, the system continues to move around the cycle, the concentrations of the chemical species continually revisit values they have had before, and the system exhibits oscillations.

Degradation stabilizes protein levels for a constitutively expressed protein. For a protein P whose synthesis is unregulated

$$\frac{dP}{dt} = k - d_P P \tag{5.1}$$

and at steady-state when $P = P^*$ the rate of synthesis, k, exactly equals the rate of degradation

$$k = d_P P^*. \tag{5.2}$$

If levels of P fluctuate higher than P^*, then the rate of synthesis is unchanged but the rate of degradation increases, $d_P P > d_P P^*$, so that degradation dominates synthesis. Degradation therefore returns the levels of proteins to their steady-state levels. Similarly, if levels of P fluctuate lower than P^*, then the rate of degradation decreases, $d_P P < d_P P^*$, allowing synthesis to dominate and steady-state to be regained.

Negative feedback acts to reduce perturbations to a system. Consider a gene that is negatively autoregulated by its protein P so that

$$\frac{dP}{dt} = \frac{k}{1 + (P/K)^n} - d_P P \tag{5.3}$$

where we use a Hill function to describe the autoregulation. This negative autoregulation generates negative feedback in the system. At steady-state, $P = P^*$ and

$$\frac{k}{1 + (P^*/K)^n} = d_P P^* \tag{5.4}$$

so that the rate of synthesis of P balances its rate of degradation. If levels of P fluctuate higher than P^*, then

$$\frac{k}{1 + (P/K)^n} < \frac{k}{1 + (P^*/K)^n} \tag{5.5}$$

and the autonegative control increases repression of synthesis (there are more proteins to bind the promoter of P) and so decreases levels of P back to P^*. If levels of P fluctuate lower than P^*, then

$$\frac{k}{1 + (P/K)^n} > \frac{k}{1 + (P^*/K)^n} \tag{5.6}$$

and the autonegative control lessens (there are less proteins to bind the promoter of P) increasing synthesis and resulting in an increase P towards P^*. The autonegative control is therefore a negative feedback and provides an additional restoring process to degradation.

Delayed negative feedback, if the feedback is sufficiently strong and the delay is sufficiently long, can, however, be destabilizing and generate oscillations. Consider again a negatively autoregulated gene. If levels of protein begin to rise above average levels, then repression will

increase. If, however, there is a delay before the increase in repression then levels will rise higher than for a system without a delay. Once repression does act, there is a mismatch between the strength of repression, which is determined by levels of protein that existed a delay time earlier, and the current levels of protein. Protein levels are not therefore brought back to their average values but undershoot the average because repression is too strong. If there is also a delay in releasing repression then when levels of proteins return to the average, the strength of repression is again mismatched, but is too weak this time. Protein levels consequently overshoot the average value, and a cycle initiates. The delays in negative feedback cause protein levels to alternatively undershoot and overshoot their average level, and the system oscillates.

5.1 Circadian rhythms

Circadian rhythms are free-running oscillations generated by biochemical networks within single cells. By free-running, we mean they can exist in the absences of cues from the earth's 24 hour cycle. Circadian rhythms have a period of approximately 24 hours and can be synchronized by environmental signals, such as light and temperature. They are also temperature compensated meaning that they can persist over a range of temperatures.

Circadian rhythms have been studied in *Drosophila* and the first mutation to disrupt the circadian rhythm was discovered in flys by Konopka and Benzer. This mutation was in the *period* or *per* gene.

In *Drosophila*, the basis of the circadian rhythm is delayed negative feedback through negative autoregulation of the *per* gene by the PER protein. After transcription, PER proteins accumulate in the cytoplasm and re-enter the nucleus only after a delay to repress transcription of *per*. PER is phosphorylated in the cytoplasm by a kinase called DBT (double-time), and this phosphorylation is a requirement for PER's degradation. PER can, however, exist as both a monomer and a dimer, and DBT phosphorylates both. Only the dimer represses the *per* gene.

We will follow the model of Tyson *et al.* [12] (Fig. 12) to explore how oscillations are generated. Tyson *et al.* assume that the PER dimer rapidly equilibriates between the cytoplasm and the nucleus and that the interconversion of PER monomers and dimers is also at equilibrium.

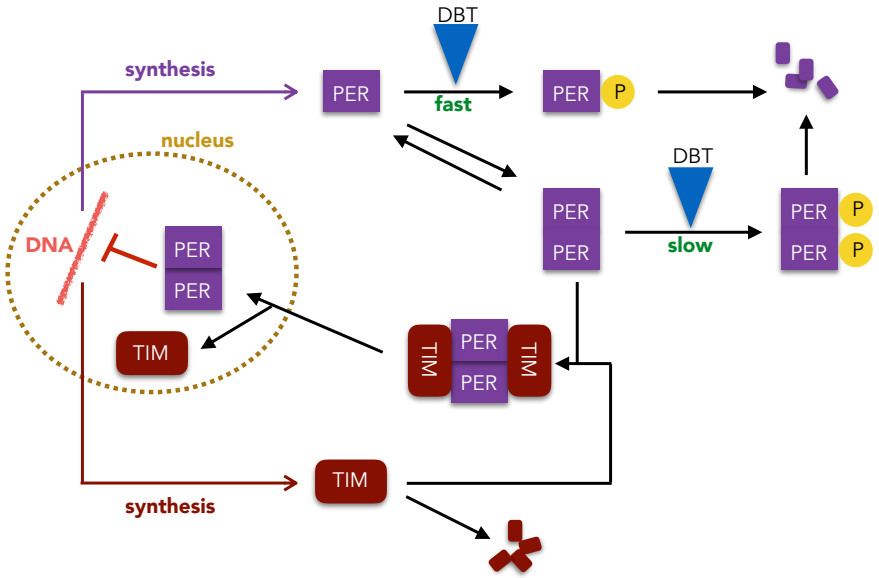


Figure 12: The model of circadian rhythms of Tyson *et al.* involves a delayed negative feedback where the transcription factor PER represses its own expression when a dimer and when imported into the nucleus in a complex with TIM. They choose, however, not to explicitly model TIM, but include its effects by having different rates of degradation of PER monomers and dimers by DBT.

5.1.1 Competitive inhibition

First, we will investigate the rate of phosphorylation of DBT, which is an enzyme that has two substrates: PER monomers and PER dimers. Assuming that both phosphorylations procede with Michaelis-Menten reactions and, denoting P_1 for PER monomers and P_2 for PER dimers, we have

$$\mathrm{D} + \mathrm{P}_1 \underset{b_1}{\overset{f_1}{\rightleftharpoons}} \mathrm{C}_1 \xrightarrow{k_1} \mathrm{P}_1^* + \mathrm{D}$$

and

$$\mathrm{D} + \mathrm{P}_2 \underset{b_2}{\overset{f_2}{\rightleftharpoons}} \mathrm{C}_2 \xrightarrow{k_2} \mathrm{P}_2^* + \mathrm{D}$$

where the kinase DBT is denoted D.

As before (see Eq. 2.60), we assume that both C_1 and C_2, the kinase-substrate complexes, are at quasi-steady-state. Then

$$\frac{dC_1}{dt} = f_1 D P_1 - (b_1 + k_1) C_1 \simeq 0$$
$$\frac{dC_2}{dt} = f_2 D P_2 - (b_2 + k_2) C_2 \simeq 0$$

and therefore

$$C_1 \simeq \tfrac{f_1 D P_1}{b_1 + k_1} \quad ; \quad C_2 \simeq \tfrac{f_2 D P_2}{b_2 + k_2}. \tag{5.7}$$

The total amount of kinase, D_T, is fixed, and $D + C_1 + C_2 = D_T$. This conservation law with Eq. 5.7 implies that

$$D = \frac{D_T}{1 + \frac{f_1 P_1}{b_1 + k_1} + \frac{f_2 P_2}{b_2 + k_2}}. \tag{5.8}$$

Consequently, the rate of formation of P_1^*, which is $k_1 C_1$, equals

$$k_1 \times \frac{f_1 P_1}{b_1 + k_1} \times \frac{D_T}{1 + \frac{f_1 P_1}{b_1 + k_1} + \frac{f_2 P_2}{b_2 + k_2}} \tag{5.9}$$

using Eqs. 5.7 and 5.8. We can thus write

$$\frac{dP_1^*}{dt} = \frac{k_1 D_T P_1}{\frac{b_1 + k_1}{f_1} + P_1 + \frac{f_2(b_1 + k_1)}{f_1(b_2 + k_2)} P_2} \tag{5.10}$$

and similarly can show that the rate of formation of P_2^* is

$$\frac{dP_2^*}{dt} = \frac{k_2 D_T P_2}{\frac{b_2 + k_2}{f_2} + P_2 + \frac{f_1(b_2 + k_2)}{f_2(b_1 + k_1)} P_1}. \tag{5.11}$$

PER dimers therefore inhibit the phosphorylation of PER monomers (high P_2 decreases dP_1^*/dt) and PER monomers inhibit the phosphorylation of PER dimers (high P_1 decreases dP_2^*/dt). Both isoforms are competitive inhibitors of each other.

If the Michaelis-Menten constant of DBT is the same for both substrates, so that $\frac{b_1 + k_1}{f_1} = \frac{b_2 + k_2}{f_2} = K$ say, then

$$\begin{aligned} \frac{dP_1^*}{dt} &= \frac{V_1 P_1}{K + P_1 + P_2} \\ \frac{dP_2^*}{dt} &= \frac{V_2 P_2}{K + P_1 + P_2} \end{aligned}$$

with $V_1 = k_1 D_T$ and $V_2 = k_2 D_T$. Eq. 5.1.1 is the form used by Tyson *et al.* [12].

5.1.2 Dimerization

Second, we investigate the equilibrium properties of the dimerization reaction of PER proteins. This reaction is

$$\mathrm{P}_1 + \mathrm{P}_1 \underset{b}{\overset{f}{\rightleftharpoons}} P_2$$

and, by mass action, generates the following dynamics for P_1

$$\frac{dP_1}{dt} = -2 f P_1^2 + 2 b P_2 \tag{5.12}$$

where the factor of two arises because the forward reaction decreases the number of P_1 molecules by two and the backward reaction increases the number of P_1 molecules by two. The dimer obeys

$$\frac{dP_2}{dt} = f P_1^2 - b P_2. \tag{5.13}$$

At equilibrium,

$$P_2 = \frac{f}{b} P_1^2 \tag{5.14}$$

and if we let

$$P_T = P_1 + 2 P_2 \tag{5.15}$$

then

$$P_T = P_1 + 2\frac{f}{b}P_1^2 \tag{5.16}$$

which is a quadratic equation:

$$P_1^2 + \frac{b}{2f}P_1 - \frac{b}{2f}P_T = 0 \tag{5.17}$$

This equation can be solved following the usual formula

$$\begin{aligned} P_1 &= \frac{-1 + \sqrt{1 + 8\frac{f}{b}P_T}}{4\frac{f}{b}} \\ &= \frac{2P_T}{1 + \sqrt{1 + 8\frac{f}{b}P_T}} \end{aligned}$$

where we have multiplied both top and bottom by $2/q$ where q is

$$q = \frac{2}{1 + \sqrt{1 + 8\frac{f}{b}P_T}} \tag{5.18}$$

Consequently, we can write the equilibrium concentrations in the convenient form

$$P_1 = qP_T \quad ; \quad P_2 = \tfrac{1}{2}(1-q)P_T \tag{5.19}$$

with q given by Eq. 5.18.

5.1.3 The Tyson *et al.* model

Tyson *et al.* have three equations in their model: one for *per* mRNA, one for PER monomers, and one for PER dimers. They use a Hill function with a Hill number of two to model negative autoregulation of *per* expression by PER dimers. The equation for *per* mRNA levels is then

$$\frac{dM}{dt} = \frac{u}{1 + \frac{P_2^2}{P_c^2}} - d_M M \tag{5.20}$$

with d_M being the rate of degradation of mRNA.

PER monomers are translated from the mRNA with rate v, are phosphorylated by DBT, are actively degraded at rate d_p, and undergo dimerization:

$$\frac{dP_1}{dt} = vM - \frac{V_1 P_1}{K + P_1 + P_2} - d_P P_1 - 2fP_1^2 + 2bP_2 \tag{5.21}$$

using Eq. 5.1.1. Once phosphorylated, the PER monomers are assumed to rapidly degrade and no longer play any part in the dynamics.

PER dimers also undergo phosphorylation, degradation, and monomerization:

$$\frac{dP_2}{dt} = -\frac{V_2 P_2}{K + P_1 + P_2} - d_P P_2 + fP_1^2 - bP_2. \tag{5.22}$$

Once phosphorylated, the PER dimers rapidly degrade too and are no longer relevant for the dynamics.

By assuming equilibrium between PER monomers and dimers, Tyson *et al.* were able to reduce this system of three equations to two equations. By adding dP_1/dt to twice dP_2/dt, they found an equation for $P_T = P_1 + 2P_2$:

$$\frac{dP_T}{dt} = vM - \frac{V_1 q + V_2(1-q)}{K + \frac{1}{2}(1+q)P_T} P_T - d_P P_T \tag{5.23}$$

using Eq. 5.19. Similarly, we use Eq. 5.19 to write the equation for mRNA in terms of P_T rather than P_2:

$$\frac{dM}{dt} = \frac{u}{1 + \frac{(1-q)^2 P_T^2}{4P_c^2}} - d_M M \tag{5.24}$$

with q obeying Eq. 5.18.

Eqs. 5.23 and 5.24 can be investigated using phase plane analysis. The nullclines intersect at one point, but this point is unstable for certain values of the parameters [12], and the system oscillates. The system has negative feedback because of the repression of the *per* gene by PER dimers, and this feedback is delayed because PER must be synthesized and then converted into dimers before repressing transcription. The delayed negative feedback is the basis of the circadian oscillations.

The system also has positive feedback. If the number of dimers is such that the rate of phosphorylation of dimers by DBT is saturated, then an increase in the number of dimers cannot affect the rate of phosphorylation of dimers, but does still decrease the rate of phosphorylation of PER monomers. PER monomers consequently build up and so too do PER dimers because of the equilibrium that exists between monomers and dimers. An increase in PER dimers therefore generates a further increase in dimers: the system has positive feedback. Positive feedback is strongest for dimers rather than monomers because $V_2 \ll V_1$ for this system. The feedback allows PER dimers to build up quickly once their numbers become sufficiently high and the positive feedback dominates.

5.2 Relaxation oscillations

Systems with both positive and negative feedback can undergo relaxation oscillations. A relaxation oscillation has a slow buildup, where we can think of 'stress' being accumulated, and then a fast 'discharge', where the stress is relaxed. The system must there have two widely separated time scales [11].

The Tyson *et al.* model has positive and negative feedback and both slow and fast times scales, and it exhibits relaxation oscillations. There is a slow time scale because the numbers of PER proteins only gradually increase because mRNA must first by synthesized and then translated. Once PER dimers repress transcription of *per* and the levels of mRNA start to decrease then the source of PER monomers (through translation of the mRNA) is lost. The fast time scale arises because the numbers of PER molecules quickly fall because PER monomers are degraded through the action of the kinase DBT more rapidly than PER dimers ($V_1 \gg V_2$ in Eqs. 5.20 and 5.21). As PER monomers are lost, PER dimers dissociate into monomers to maintain the dimer-monomer equilibrium and so provide more substrate for DBT and further loss of PER.

5.3 Oscillations through both positive and negative feedback

Oscillators with both positive and negative feedback are typically built around a bistable system that exists if there is no negative feedback. For example, if there is no repression of *per* transcription by PER dimers in the Tyson *et al.* model then the system no longer oscillates but becomes bistable with two stable steady-states. Tyson *et al.* postulate that the circadian oscillator may have evolved from a bistable system, which switches 'on' with dawn and 'off' with dusk assuming that a component of the network can be regulated by light (they choose the dissociation constant of dimerization of PER) [12]. A clock improves on a switch because the cell can prepare for the day in advance without needing activation by light.

With both positive and negative feedback, the limit cycle driving the oscillations is often built around the hysteretic loop generated by the underlying bistability. Negative feedback causes this bistability to be lost, and the system to oscillate, but the properties of those oscillations are still determined by the former hysteretic loop. The dynamics will be slow when the concentrations of the oscillating species are near the steady-state values of the underlying bistable system and fast when the concentrations of the oscillating species are far from these values. The amplitude of the oscillations is approximately determined by the difference in concentrations between the two steady-states of the bistable system, and their frequency is determined by the time taken by the system to move around the loop.

Such relaxation oscillators can have a frequency and amplitude that are robust to stochastic fluctuations [13]. The frequency is more robust if stochastic fluctuations tend to be higher when the oscillator is moving quickly (for example, numbers of molecules are low then) because the period is comprised mostly of the time when the oscillator moves slowly [14]. The amplitude is more robust because it is determined by the underlying stable fixed points of the bistability [13, 15].

These underlying fixed points also allow dual feedback oscillators to tune the frequency of the oscillations while maintaining their amplitude [15]. Such behaviour is, for example, important for the effective functioning of the human heart. As the frequency changes, the underlying fixed points need not. For most systems, however, the limit cycle of the oscillations does not completely follow the hysteretic loop and so the amplitude can change as the frequency changes, but usually this change is smaller than the equivalent change in amplitude for an oscillator built from negative feedback alone [15].

Appendix A Fitting data

Often we want to fit a mathematical model to data, and here we will briefly discuss how to do so. For illustration, we will consider a simple, one-parameter model for a protein A that is degraded at a rate k and we wish to infer k from a data set. The differential equation describing the model is

$$\frac{dA}{dt} = -kA \tag{A.1}$$

and, as well as the parameter k, there is an additional parameter: the initial number of A molecules, denoted A_0.

We will use a Bayesian approach. In Bayesian probability theory, the probability of an event is interpreted as the degree of belief in that event: the higher the probability, the more confident we are that the event will or has occurred [16].

Inference uses Bayes's rule to update our prior (initial) belief to our posterior belief based on the data that has been observed. In the context of parameter fitting, prior beliefs are typically some range in which we believe the parameter exists, for example, the positive numbers or between some minimum and maximum values. For the parameter k and a data set, D, Bayes's rule is (the symbol | is read as 'given') [16]

$$\begin{aligned} P(k|D) &= \frac{P(D|k)P(k)}{P(D)} \\ &\propto P(D|k)P(k) \end{aligned}$$

where $\propto$ means proportional to: we need not be concerned with the denominator because $P(D)$ is independent of the parameter k. The probability $P(k)$ is the prior probability of k; the probability $P(D|k)$ is known as the likelihood; and the probability $P(k|D)$ is the posterior probability of k. As we define the prior probability based on our initial knowledge, the likelihood is the only quantity that must be calculated.

For our problem with two unknowns, Bayes's rule becomes

$$P(k, A_0|D) \propto P(D|k, A_0)P(k, A_0) \tag{A.2}$$

and we have to calculate the likelihood given values for k and A_0 consistent with the prior probability.

To calculate the likelihood, we need an explicit model of the the measurement error. Usually, the measurement error, ϵ, is assumed to be identically, independently, and normally distributed with a mean of zero and a standard deviation σ, which determines its typical size:

$$P_{\mathrm{e}}(\epsilon) = \frac{\exp\left(\frac{-\epsilon^2}{2\sigma^2}\right)}{\sqrt{2\pi}\sigma} \tag{A.3}$$

If d_i is the data point measured at time t_i and A_i is the corresponding predicted value, found by integrating Eq. A.1, then d_i and A_i are related through the measurement error at that time point, ϵ_i:

$$d_i = A_i + \epsilon_i \tag{A.4}$$

or

$$d_i - A_i = \epsilon_i. \tag{A.5}$$

Note that the predicted value A_i depends on the values chosen for the parameters k and A_0 (values are needed to, for example, numerically integrate Eq. A.1). Using Eq. A.3, we can write

$$\begin{aligned} P(d_i|k, A_0) &= P(d_i|A_i) \\ &= P_{\mathrm{e}}(d_i - A_i) = \frac{\exp\left(\frac{-(d_i-A_i)^2}{2\sigma^2}\right)}{\sqrt{2\pi}\sigma} \end{aligned}$$

assuming that the value of σ is known in advance and is part of our prior information.

For the complete likelihood, the error in each data point is assumed to be independent of the error in any other data point, which means that

$$\begin{aligned} P(D|k, A_0) &= P(d_0, \ldots, d_n|k, A_0) \\ &= P(d_0, \ldots, d_n|A_0, A_1, \ldots, A_n) \\ &= P(\epsilon_i, \ldots, \epsilon_n) \\ &= P_{\mathrm{e}}(\epsilon_i) \ldots P_{\mathrm{e}}(\epsilon_n) \end{aligned}$$

where we assume n data points. Using Eq. A, Eq. A is

$$\begin{aligned} P(D|k, A_0) &= \prod_{i=0}^{i=n-1} \frac{\exp\left(\frac{-(d_i-A_i)^2}{2\sigma^2}\right)}{\sqrt{2\pi}\sigma} \\ &= \frac{\exp\left(-\sum_{i=0}^{n-1} \frac{-(d_i-A_i)^2}{2\sigma^2}\right)}{\left(\sqrt{2\pi}\sigma\right)^n} \end{aligned}$$

which is the complete expression for the likelihood remembering that the A_i are the predictions of the model at times t_i and need to be found typically through numerically integration.

Eq. A with the prior distribution, $P(k, A_0)$, and Eq. A.2 allows the posterior probability of k and A_0 to be calculated. Figs. 13 and 14 show two example calculations of the posterior probabilities. Although the posterior probability itself is the complete result of the inference, often we wish to give a 'best-fit' value for the parameters. These 'best-fit' values are the values of the parameters corresponding to the peaks (the modes) of the corresponding posterior distributions and the errors in the inference are given by the width of the posterior distributions at these peaks.

Numerical tricks

When calculating probabilities, often numbers can be small, and there are a few tricks to avoid underflow errors (numbers too small for the computer to accurately store).

First, typically the negative logarithm of the likelihood is calculated. Taking the logarithm of Eq. A, this 'energy', as it is sometimes called in analogy with approaches from physics, is

$$\mathcal{E} = \sum_{i=0}^{n-1} \frac{(d_i - A_i)^2}{2\sigma^2} + n\log(\sqrt{2\pi}) + n\log(\sigma) \tag{A.6}$$

and the most likely values of the parameters are the ones that maximize the likelihood and so minimize the energy. The energy is a sum of squares, which justifies the 'sum of squares' approaches that are often used in fitting.

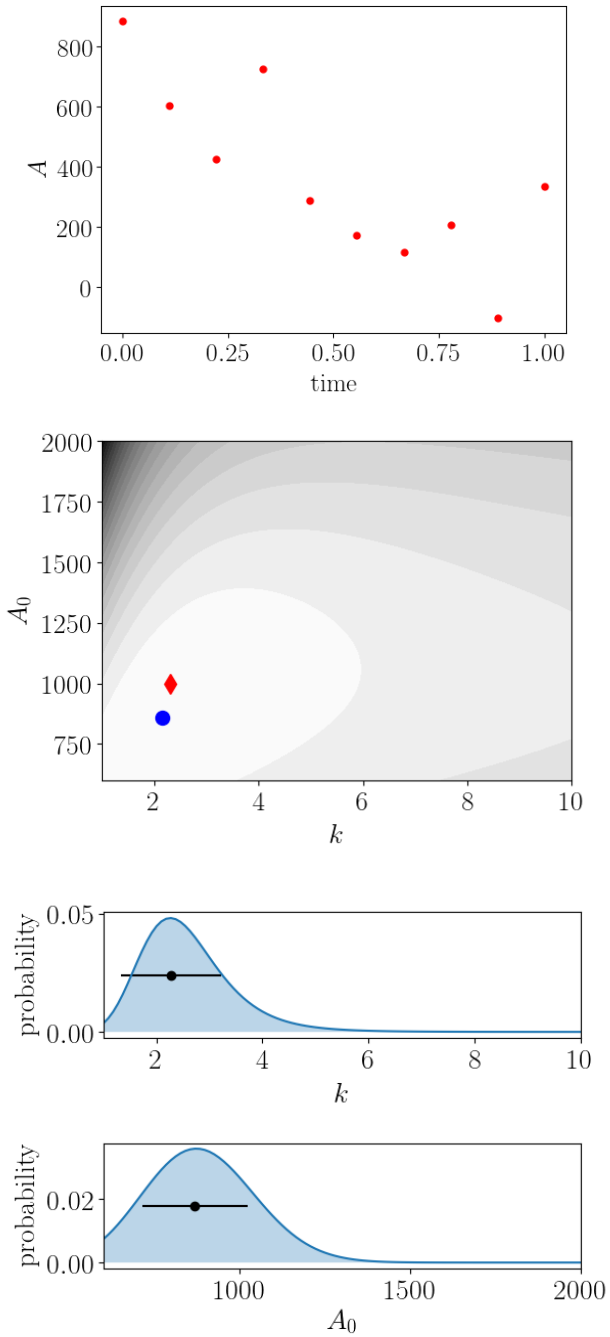


Figure 13: Inference with a few data points ($n = 10$). The data (top), the log posterior probability (middle with the maximum posterior probability marked as a blue dot and the true values of $k = 2.3$ and $A_0 = 1000$ marked with a red diamond), and the posterior probabilities for k and A_0 (bottom) are shown (determined by summing the posterior probability over either A_0 or k values), with the best estimates and their associated errors.

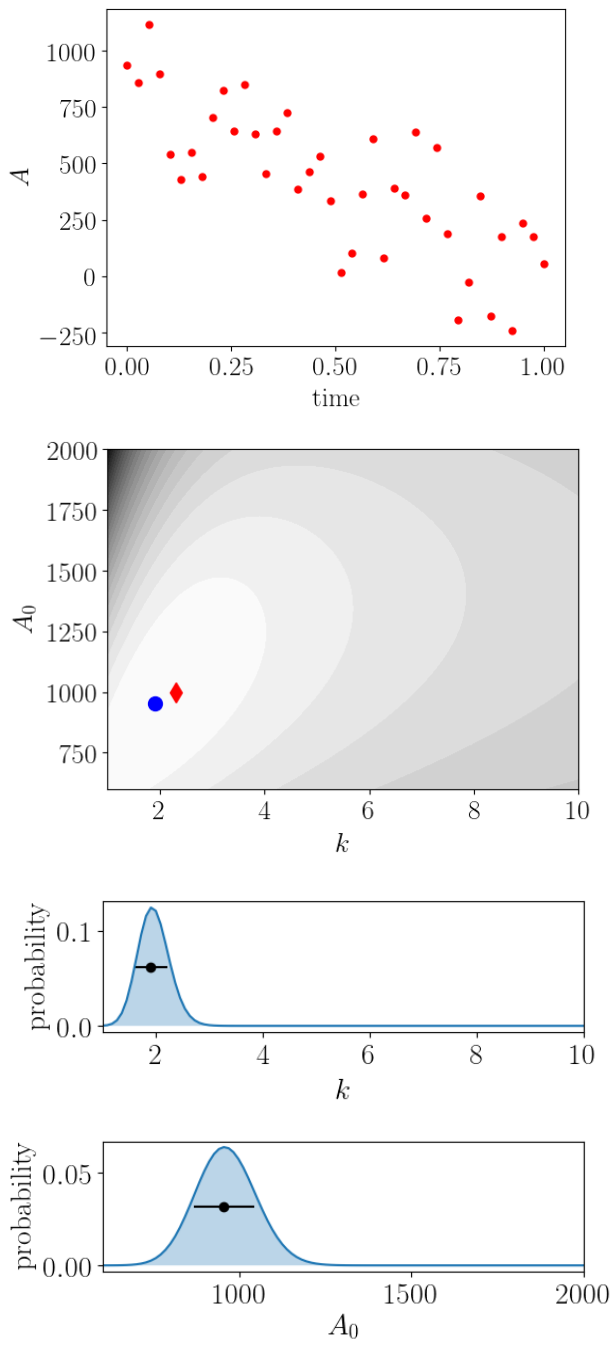


Figure 14: Inference improves with more data ($n = 40$). The data (top), the log posterior probability (middle), and the posterior probabilities for k and A_0 (bottom) are shown. Notice how the posterior probabilities have tightened.

Second, we often wish to find the most probable values of the parameters and so numerically would like to find the values of the parameters that maximize the posterior probability or, equivalently for suitable prior distributions, minimize the energy. Parameter values are typically positive in systems biology, but numerical optimization schemes may not allow this bound to be imposed. A trick is to transform the parameters

$$k = \exp(\tilde{k}) \quad ; \quad A_0 = \exp(\tilde{A}_0) \tag{A.7}$$

and minimize the energy as a function of $\tilde{k}$ and $\tilde{A}_0$. If the optimization causes either of these transformed parameters to become negative, then k and A_0 are still positive from Eq. A.7.

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