# Practical Systems Biology

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# **Contents**





# <span id="page-2-0"></span>1 Overview

These notes from the basis of lectures given to MSc students in the School of Biological Sciences at the University of Edinburgh.

In Sections [2](#page-3-0) and [3,](#page-27-0) 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\)](#page-2-1). 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\)](#page-37-0). Negative feedback can cause oscillations and drives biological rhythms (Sec. [5\)](#page-45-0).

<span id="page-2-1"></span>

Figure 1: An idealised 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 produced by this gene,  $G$ , is the system's output.

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In the association rate,  $f$ , is determined by two times: the time taken by a molecule of  $A$  and a molecule of  $B$  to find each other by diffusion,  $t_{\text{diff}}$ , and the time taken for the two molecules to react once in physical proximity,  $t_{\text{reac}}$ . We can write<br>time of reaction =  $t_{\text{diff}} + t_{\text{reac}}$  (2.1)  $\alpha$  morecute of  $D$  to find each other by diffusion,  $t_{\text{diff}}$ ,  $\alpha$  to react once in physical proximity,  $t_{\text{reac}}$ . We can write d Res<br>|-<br>|- $\frac{d}{d\cos \theta}$ a molecule of B to find each other by diffusion,  $t_{\text{diff}}$ , and the time taken for the two molecules<br>to react once in physical proximity,  $t_{\text{reac}}$ . We can write reaction. Although the association rate  $\tilde{f}$  is determined by two times; the time telen by a molecule of A and The association rate,  $\hat{f}$ , is determined by two times: the time taken by a molecule of A and

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and so the association rate, which is inversely related to the time of the reaction, obeys\n
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\tilde{f} = (t_{\text{diff}} + t_{\text{reac}})^{-1} \,. \tag{2.2}
$$

and so the association rate, which is inversely related to the time of the reaction, obeys<br>  $\tilde{f} = (t_{\text{diff}} + t_{\text{reac}})^{-1}$ . (2.2)<br>
The dissociation rate,  $\tilde{b}$ , is determined by one time — the half-life of the molecule

The dissociation rate, 
$$
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$$
, is determined by the time – the matrix of the molecule  $\sigma$ .  

$$
\tilde{b} = \frac{\log 2}{\text{half-life of } C}.
$$
 (2.3)

 $\sigma = \frac{1}{\text{half-life of } C}$ . (2.3)<br>We wish to describe how the number of C molecules, N<sub>C</sub>, 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 associa  $\tilde{b}dtN_C$ . Therefore, the number of C molecules at a time  $t + dt$  is the number of C molecules at the association and dissociation reactions will both occur (we will include<br>ter) The number of pairs of A and B molecules is  $N_A N_B$  and  $\tilde{f} dt N_A N_B$  of bative. Therefore, the number of C molecules at a time  $t + at$  is the number of C molecules at time t plus the number gained in association reactions and minus the number lost in dissociation 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  $\delta dt N_C$ . Therefore, the n we wish to describe now the humber of C molecules,  $N_C$ , changes with time. Over a small<br>include<br>interval of time dt, the association and dissociation reactions will both occur (we will include ince associated tote, 1). The accomolation of two times the turn to take molecule of A and<br>solution of the both reactions. We can turn the time taken for the two melocules<br>in react once in physical proximity,  $i_{\text{max}}$ . W nd<br>
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a molecule of B to find each other by diffusion,  $t_{\text{min}}$  and the time taken for the two molec reaction proceeds at the rate <sup>f</sup> <sup>R</sup>[ these pairs will associate over a time dt. The number of C molecules that dissociate over dt is  $\tilde{b}dtN_C$ . Therefore, the number of C molecules at a time  $t + dt$  is the number of C molecules at reactions:

<span id="page-3-3"></span><span id="page-3-2"></span>
$$
N_C(t + dt) = N_C(t) + \tilde{f}dtN_AN_B - \tilde{b}dtN_C
$$
\n(2.4)

or

$$
N_C(t + dt) = N_C(t) + fdtN_AN_B - bdtN_C
$$
\nor\n
$$
\frac{N_C(t + dt) - N_C(t)}{dt} = \tilde{f}N_AN_B - \tilde{b}N_C.
$$
\n(2.5)

Taking the limit of  $dt$  going to zero, we have or  $\frac{N_C(\ell)}{\ell}$  <br>Taking the limit of  $dt$  going to zero.  $\limsup$ % or Taking the limit of  $dt$ 

Taking the limit of *dt* going to zero, we have\n
$$
\frac{dN_C}{dt} = \tilde{f}N_AN_B - \tilde{b}N_C
$$
\n(2.6)

which is an example of a chemical rate equation.

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

$$
[C] = \frac{N_C}{n_A V} \tag{2.7}
$$

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. [2.6](#page-3-2) into an equation for the rate of change of the concentration of  $C$ , we must divide Eq. [2.6](#page-3-2) by  $n_A V$ . This division gives

$$
\frac{d}{dt} \cdot \frac{N_C}{n_A V} = \tilde{f} \frac{N_A}{n_a V} \cdot \frac{N_B}{n_a V} n_a V - \tilde{b} \frac{N_C}{n_A V} \tag{2.8}
$$

and so

$$
\frac{d[C]}{dt} = \tilde{f} n_A V[A][B] - \tilde{b}[C] \tag{2.9}
$$

where  $[A]$  is the concentration of A and  $[B]$  is the concentration of B.

If we define macroscopic reactions rates, or rates for reactions involving concentrations, as

<span id="page-4-1"></span>
$$
f = \tilde{f} n_A V
$$
  
\n
$$
b = \tilde{b}
$$
\n(2.10)

then

<span id="page-4-4"></span>
$$
\frac{d[C]}{dt} = f[A][B] - b[C].
$$
\n(2.11)

The units of the macroscopic association rate f are  $M^{-1}$  s<sup>-1</sup>. This rate should not change with volume because molecular species are now measured in concentrations. The rate of association of a pair of molecules,  $f$ , does, however, depend on volume and will decrease in larger volumes because it is more difficult for molecules to find each other [\[1\]](#page-63-0). The volume-dependence in Eq. [2.10](#page-4-1) cancels out the volume-dependence of  $\tilde{f}$ . In contrast, the units of the macroscopic rate b do not change, remaining  $s^{-1}$ , because b describes a dissociation reaction that depends principally on the chemical species involved and occurs at a rate independent of volume.

#### <span id="page-4-0"></span>2.1.1 Example: dimerisation

Many membrane receptors reversibly dimerise to form a receptor-receptor dimer, and transcription factors in bacteria often dimerise before binding to DNA, but the dimerisation reaction is unusual.

Let  $T$  denote a transcription factor and  $T_2$  denote a dimer of two transcription factors. These species satisfy that i pure for this system are atypical because the f reaction removes two molecules of  $T$  rather than one and the  $b$  reaction releases two molecules. Although the association reaction proceeds at the rate  $f[T]^2$  and the dissociation reaction proceeds at the rate  $b[T_2]$ , we now have

<span id="page-4-2"></span>
$$
\frac{d[T]}{dt} = -2f[T]^2 + 2b[T_2] \tag{2.12}
$$

because the number of  $T$  molecules changes by two for both reactions. The dimer,  $T_2$ , obeys

<span id="page-4-3"></span>
$$
\frac{d[T_2]}{dt} = f[T]^2 - b[T_2] \tag{2.13}
$$



because only one molecule of dimer either forms or dissociates.<br>Summing Eq. 2.12 and twice Eq. 2.13 gives converted Eq. 3 into an equation for the rate of  $\mathbb{S}\mathfrak{u}$ because only one molecule of dimer either forms or dissociates.<br>Summing Eq.  $2.12$  and twice Eq.  $2.13$  gives  $\ddot{ }$ Let  $\alpha$  and R<sup>2</sup> denote a receptor and R<sup>2</sup> denote a discovered respectively the reaction species species satisfy the reaction of dimer estimates and reactions.

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$$

<span id="page-5-1"></span>implying that ˜b

$$
\frac{d[T]}{dt} + 2\frac{d[T_2]}{dt} = 0
$$
 (2.14)  
implying that  

$$
[T] + 2[T_2] = \text{constant} = [T]_0 + 2[T_2]_0
$$
 (2.15)  
where  $[T]_0$  is the initial concentration of monomers and  $[T_2]_0$  is the initial concentration of

where  $\lfloor I \rfloor_0$  is the initial concentration of monomers and  $\lfloor I_2 \rfloor_0$  is the initial concentration of dimers.  $\theta$  of  $f$  $\frac{1}{\pi}$  is the initi  $\frac{d[T]}{dt} + 2\frac{d[T]}{dt}$ <br>  $[T] + 2[T_2] = \text{constant}$ <br>
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The dimerisation reaction only changes the form of  $T$  molecules, either from monomers to dimers or vice versa, and does not lead to either their synthesis or destruction. Consequently, the number of  $T$  molecules is cons dimers or vice versa, and does not lead to either their synthesis or destruction. Consequently,<br>the number of  $T$  molecules is conserved and determined by the initial numbers of monomers<br>and dimers. The conservation law,  $\frac{1}{\sqrt{2}}$ because only one molecule of dime<br>
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If we have  $N_0$ <br> summing Eq. 2.12 and vote Eq. 2.43 are unchanged and  $\frac{dV_1}{dt} + 2\frac{d^2V_2}{dt} = 0$  (2.14)<br>
implying that  $[Y]_1 + 2[x]_2] = \text{constant} = [T]_0 + 2[x]_0$ . (2.15)<br>
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\cdot\n\end{array}$ **because the cause of the dimersion of the dimersio because only one molecule of the molecule of dimer contents of dimer state of**  $\frac{d[1]}{dt} + 2\frac{d[T_2]}{dt} = 0$  **(2.14)<br>
[7] + 2[73] = constant = [7]<sub>0</sub> + 2[73]<sub>0</sub> (2.15)<br>
necentration of monomers and [73]<sub>0</sub> is the initial c** al cor<br>The rations for the rate of the power of the power of the rate of the r reaction and two molecules are released by the set of models are released by the release of models are released by the release because only only ing the molecule:<br>Because of the control of the dimer forms of dimer forms or dissociate<br>. 1 and twice Eq. 9  $T_{\text{in}}$  is the either either either and d<br>  $1.15$ , r<br>  $T_{\text{out}}$  = has t<br>  $\frac{dN}{dt}$  = has t<br>  $\frac{dN}{dt}$  = (e<sup>1</sup><br>  $= 2\frac{1}{16}$ <br>  $= N_0$ <br>  $(6)$ 1.1.1 Example: dimerization Many membrane receptors reversible receptors reversible to form a receptor-receptor-receptor-receptor-receptor-receptor-receptor-receptor-receptor-receptor-receptor-receptor-receptor-receptor-receptor-receptor-receptor-rec entrat only the dimersion of the dimersion  $\mathbf{a}$  and signal down is unusual. ners<br>1v  $\overline{T}$  $\alpha$  is or vice versa, and does not lead to either their synthesis or destruction. Consequently, 2.14)<br>2.15)<br>n of<br>cs to<br>mtly, molecules as a monomer. s to<br>tly,<br>y T al cor<br>The rate equations for the rate of the rate of the rate at this control<br>and the cules of the power reaction and two molecules are released by the set of models are released by the release of models are released by the release of the release of the released by the released by the released by the released by the released  $\begin{align*} \mathbb{E}[T_2]_0 \text{ is} \end{align*}$  and the rate discontinuous proceeds at the ration process at the ration process at the rate of  $N$  observable sum  $N$ .<br>(  $P_{\text{max}}$  and  $\text{max}$  are  $P_{\text{max}}$  and  $P_{\text{max}}$  and  $P_{\text{max}}$ molecules as a monomer.<br> there is or vice versa, and does not lead to enter the number of  $T$  molecules is conserved and because only only ing the controller or dimer forms or dimer forms or dissociate or disconcile or disconciled the disconciled twice the disconcern or disconcern twice  $\mathbf{r}$  and  $\mathbf{r}$  and  $\mathbf{r}$  and  $\mathbf{r}$  and  $\math$ and dimers. The conservation law, Eq.  $2.15$ , reflects that a dimer contains twice as many  $T$ ׇׅׅ֘֒֝֬ or diss<br>  $\frac{T_2}{lt}$  =  $\frac{1}{lt}$ <br>
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# <span id="page-5-0"></span>2.1.2 Rates of first-order reactions 2.1.2

2.1.2 Rates of first-order reactions<br>
In Eq. 2.3, there is a log 2 term. This term comes from the definition of half-life: the average<br>
time taken for the num[be](#page-3-3)r and so also typically the concentration of molecules to hal 2.1.2 Rates of first-order reactions<br>In Eq. 2.3, there is a log 2 term. This term comes from the definition of half-life: the average<br>time taken for the number, and so also typically the concentration, of molecules to hal time taken for the number, and so also typically the concentration, of mole[cule](#page-5-2)s to halve. If a<br>molecule degrades at a rate k, then the number of molecules N obeys<br> $\frac{dN}{dt} = -kN.$  (2.16)<br>If we have  $N_0$  molecules initial **2.1.2 Rates of first-order reactions**<br>
In Eq. 2.3, there is a log 2 term. This term comes time taken for the number, and so also typically the<br>
molecule degrades at a rate k, then the number of n<br>  $\frac{dN}{dt} = -kl$ <br>
If we ha time taken for the number, and so also typically the concentration, of molecules to halve. If a molecule degrades at a rate  $k$ , then the number of molecules  $N$  obeys  $\det$  in the ration  $N$  obe<br>  $\det$  at  $\det$  and  $\det$  is so rage If a<br>2.16)<br>2.17)<br>ising<br>2.18)  $\frac{1}{\text{the}}$  cos from  $\frac{d}{d\theta}$  to the contractions depend on  $\frac{d}{d\theta}$  and  $\frac{d}{d\theta}$ molecule degrades at a rate  $k,$  then the number of molecules  $N$  obeys  $\frac{dN}{dt} = -kN.$ In Eq. 2.3, there is a log 2 term. This term comes from the definition of half-life: the average  $\overline{\phantom{a}}$ 

molecule degrades at a rate 
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, then the number of molecules *IN* oveys  
\n
$$
\frac{dN}{dt} = -kN.
$$
\n(2.16)  
\nIf we have  $N_0$  molecules initially, Eq. 2.16 has the solution  
\n $N = N_0 e^{-kt}$ , (2.17)  
\nand the number of molecules decreases exponentially with time.  
\nTo determine the molecule's half-life, we should re-write this solution in powers of 2. Using  
\nthe mathematical relation for any variable a  
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e^a = (e^{\log 2})^{\frac{a}{\log 2}}
$$
\n
$$
= 2^{\frac{a}{\log 2}}
$$
\nwe can write Eq. 2.17 as  
\n
$$
N = N_0 2^{-\frac{kt}{\log 2}}.
$$
\n(2.18)

<span id="page-5-3"></span><span id="page-5-2"></span>If we have  $N_0$  molecules initially, Eq. 2.16 has the solution

If we have 
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 molecules initially, Eq. 2.16 has the solution  
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N = N_0 e^{-kt},
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\n(2.17) and the number of molecules decreases exponentially with time.

<span id="page-5-4"></span>and the number of molecules decreases exponentially with time.

The molecule's half-life, we should re-write this solution in powers of 2. Using cal relation for any variable  $a$ the m[at](#page-5-3)hematical relation for an  $% \left( \beta \right)$  we can write Eq. 2.17 as If we have  $N_0$  molecules in and the number of moleculto To determine the moleculation we can write Eq. 2.17 as  $\text{res of } 2.$  Using % eases exponentially with time. <br> alf-life, we should re-write this solution in powers variable<br>  $\alpha$ powers of  $\overline{z}$ .  $\overline{z}$ reaction proceeds at the rate  $\frac{1}{2}$ .17)<br>ising<br>2.18)  $N = N_0 e^{-kt}$ , (2.17)<br>the number of molecules decreases exponentially with time.<br>To determine the molecule's half-life, we should re-write this solution in powers of 2. Using  $1.1$  Example: dimerization  $\frac{1}{10}$ the mathematical relation for any variable a

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\frac{dN}{dt} = -kN.
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e^a = (e^{\log 2})^{\frac{a}{\log 2}}
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= 2^{\frac{a}{\log 2}}
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\nwe can write Eq. 2.17 as  
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$$
N = N_0 2^{-\frac{kt}{\log 2}}.
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in molar units (number of  $\mathcal{L}$  denote the moles of a substance per litre). % we can write Eq. 2.17  $\,$ we can wi we can write Eq. 2.17 as

$$
e^{a} = (e^{\log 2})^{\frac{a}{\log 2}}
$$
  
=  $2^{\frac{a}{\log 2}}$   
we can write Eq. 2.17 as  

$$
N = N_0 2^{-\frac{kt}{\log 2}}.
$$
 (2.18)

After a period of time equal to the half-life,  $t_{\frac{1}{2}}$ , has passed, the number of molecules will be, by definition,  $N_0/2$ . Therefore Eq. [2.18](#page-5-4) implies

$$
N_0 2^{-1} = N_0 2^{-kt_{\frac{1}{2}}/\log 2}
$$
\n(2.19)

or, comparing the exponents,

$$
1 = \frac{kt_{\frac{1}{2}}}{\log 2},\tag{2.20}
$$

which is Eq. [2.3.](#page-3-3)

#### <span id="page-6-0"></span>2.1.3 Diffusion-limited reactions

Association rates are expected to be less than  $\simeq 10^9$  M<sup>-1</sup> s<sup>-1</sup>. All association reactions proceed by the two reactants first finding each other and then reacting. We may estimate the fastest rate at which such association reactions can possibly proceed by assuming that the reactants react immediately once together, so that  $t_{\text{reac}} = 0$  in Eq. [2.2.](#page-3-4) The upper bound on association reactions is then determined from the time taken for the two reactants to diffuse together  $(t_{\text{diff}})$ . Using the diffusion equation and assuming spherical reactants, this maximum rate is [\[2\]](#page-63-1) (p. 314)

<span id="page-6-2"></span>
$$
f_{\text{max}} = 4\pi Da \tag{2.21}
$$

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  $m^2 s^{-1}$ ,  $f_{\text{max}}$  in Eq. [2.21](#page-6-2) 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<sup>-1</sup> to be able to compare with standard association rates. We therefore multiply first by Avogadro's number so as to consider a mole of reactants diffusing together (similarly to Eq.  $2.10$ ) and second by  $10<sup>3</sup>$  to convert the volume units from  $m^3$  to litres:

$$
f \text{ (in M)} < f_{\text{max}} \times n_a \times 10^3. \tag{2.22}
$$

If D is 1000  $\mu$ m<sup>2</sup> s<sup>-1</sup> and so of order the diffusion constant of water [\[3\]](#page-63-2) and around 150 times larger than the typical diffusion coefficients of proteins in the cytoplasm  $[4]$ , and a is 1 nm, then

$$
f < 4\pi \times \overbrace{10^3 \times 10^{-12}}^{D \text{ in } \mathfrak{m}^2 \mathfrak{s}^{-1}} \times \overbrace{10^{-9}}^{a} \times \overbrace{6 \times 10^{23}}^{n_a} \times \overbrace{10^3}^{\text{for } \ell}
$$
\n
$$
\simeq 7.5 \times 10^9 \,\mathrm{M}^{-1} \mathfrak{s}^{-1}.
$$
\n(2.23)

#### <span id="page-6-1"></span>2.1.4 The concentration of one molecule

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

#### <span id="page-7-0"></span>2.2 Equilibrium and detailed balance

In the absence of any input of energy, chemical reactions reach an equilibrium where the average number of molecules of each species stay constant, even though reactions will still be occurring. All time derivatives are zero, and the system is defined to be at a steady state, but equilibrium is the particular steady state where a property called detailed balance also holds.

Detailed balance means the forward rate of any reaction is exactly balanced – equal to – the backward rate. For example, in the system of reactions

$$
A + B \frac{f_1}{\sum_{b_1}^{b_1} C}
$$
  

$$
D + E
$$

the concentration of C obeys

$$
\frac{d[C]}{dt} = f_1[A][B] - b_1C + f_2[D][E] - b_2[C],\tag{2.24}
$$

and at steady state we have

$$
\frac{d[C]}{dt} = f_1[A][B] - b_1C + f_2[D][E] - b_2[C] = 0.
$$
\n(2.25)

At equilibrium, the extra condition of detailed balance implies

$$
\frac{d[C]}{dt} = \overbrace{f_1[A][B]}^0 - b_1C + \overbrace{f_2[D][E]}^0 - b_2[C] = 0
$$
\n(2.26)

so that not only is  $\frac{d[C]}{dt}$  equal to zero, but also  $f_1[A][B] = b_1[C]$  so that the first reaction is balanced and  $f_2[D][E] = b_2[C]$  so that the second reaction is balanced.

Detailed balance forces the system to be at a minimum of free energy and so 'dead'. In modelling, we often assume that a system is never able to reach equilibrium so that there is always free energy to exploit. For example, if any reaction is made irreversible, it will never be balanced, and the system may reach a steady state, but this steady state will not be an equilibrium. Implicitly we are assuming a continual supply of free energy, such as ATP, which biases the reaction to work predominately in one direction. For example, there may be a coupling of the reaction in this direction to ATP's hydrolysis.

#### <span id="page-7-1"></span>2.2.1 Finding concentrations at equilibrium

As an example of solving a system at equilibrium, consider again

$$
A + B \xrightarrow{\quad f \quad} C \tag{2.27}
$$

At equilibrium, detailed balance implies

<span id="page-7-2"></span>
$$
f[A][B] = b[C] \tag{2.28}
$$

so that the rate of association of A and B equals the rate of dissociation of  $C$ . The equilibrium dissociation constant is defined as  $K_{\text{eq}} = b/f$ , and

<span id="page-8-2"></span>
$$
[A][B] = K_{\text{eq}}[C]. \tag{2.29}
$$

From the rate equations

$$
\frac{d[A]}{dt} = \frac{d[B]}{dt} = -f[A][B] + b[C] = -\frac{d[C]}{dt}
$$
\n(2.30)

we see that

$$
\frac{d[A]}{dt} + \frac{d[C]}{dt} = 0\tag{2.31}
$$

and

$$
\frac{d[B]}{dt} + \frac{d[C]}{dt} = 0\tag{2.32}
$$

implying

<span id="page-8-3"></span>
$$
[A] + [C] = A_0 \tag{2.33}
$$

and

<span id="page-8-4"></span>
$$
[B] + [C] = B_0 \tag{2.34}
$$

for some constant  $A_0$  and  $B_0$ . These conservation laws arise because each C molecule 'contains' an A molecule and a B molecule. Together with Eq. [2.29,](#page-8-2) the conservation laws, Eq. [2.33](#page-8-3) and Eq. [2.34,](#page-8-4) define the equilibrium concentrations of  $|A|, |B|$ , and  $|C|$ .

#### <span id="page-8-0"></span>2.3 The law of mass action

The law of mass action states that the rate of a reaction should depend on its stoichiometry in the same way that equilibrium constants depend on the stoichiometry. The stoichiometry of a reaction is defined as the relative numbers of reactants and products that are expended and created by the reaction. For example, for the association reaction  $A+B \to C$ , 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. [2.11](#page-4-4) and Eq. [2.28,](#page-7-2) we see that the dependence on stoichiometry is the same because 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. [2.11.](#page-4-4) Using the law of mass action, we ensure that the dynamics of our system are such that the system reaches a thermodynamically correct equilibrium.

#### <span id="page-8-1"></span>2.4 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\)](#page-2-1). We can model this activation by a binary reaction:

$$
[\mathbf{R}] + [\mathbf{S}] \xrightarrow[\mathbf{b}]{f} [\mathbf{R}^*]
$$

To allow the activated receptors to activate 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 catalyses the conversion of A to its activated form A<sup>∗</sup> .

The corresponding differential equations are

$$
\frac{d[S]}{dt} = -f[R][S] + b[R^*]
$$
\n
$$
\frac{d[R]}{dt} = -f[R][S] + b[R^*]
$$
\n
$$
\frac{d[R^*]}{dt} = f[R][S] - b[R^*]
$$
\n
$$
\frac{d[A]}{dt} = -k[A][R^*]
$$
\n
$$
\frac{d[A^*]}{dt} = k[A][R^*]
$$
\n(2.35)

but our main focus of interest is the production of A<sup>∗</sup> because A<sup>∗</sup> 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

<span id="page-9-0"></span>
$$
f[R][S] \simeq b[R^*].\tag{2.36}
$$

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

$$
R_0 = [R] + [R^*],\tag{2.37}
$$

then, using Eq. [2.36,](#page-9-0) we can show that

<span id="page-9-2"></span>
$$
[R^*] \simeq \frac{[S]R_0}{\frac{b}{f} + [S]}.\tag{2.38}
$$

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

$$
\frac{d[A^*]}{dt} \simeq \frac{k[S]R_0}{\frac{b}{f} + [S]}[A] \tag{2.39}
$$

or

<span id="page-9-1"></span>
$$
\frac{d[A^*]}{dt} \simeq \frac{k[S]R_0}{\frac{b}{f} + [S]}(A_0 - [A^*])
$$
\n(2.40)

because the number of A molecules is conserved, with a total concentration of say  $A_0$ , because A also only changes state.

Eq. [2.40](#page-9-1) 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 eventually become activated:  $[A^*] \longrightarrow A_0$ .

# 2.5 Thermodynamic cycles  $\Omega$  . The ann extracellular ligand. The ann extracellular reversion of the formula to fo 2.5 Thermodynamic cycles

A system contains a thermodynamic cycle if it has a series of states interlinked by equilibrium A system contains a thermodynamic cycle if it has a series of states intermined by equilibrium<br>reactions and where starting from any particular state the system can return to that state by passing through a series of intermediate states.<br>  $\frac{1}{2}$ 2.5 Thermodynamic cycles<br>A system contains a thermodynamic cycle if it has a series of states interlinked by equilibrium<br>reactions and where starting from any particular state the system can return to that state by nterlinked by equilibri<br>n return to that state<br>itch from a closed to red by equal to that

passing through a series of intermediate states.<br>Ion channels often undergo thermodynamic cycles. They can switch from a closed to an for channels often undergo thermodynamic cycles. They can switch from a closed to an<br>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. a retractory state. In the retractory state, the channel rarely opens but eventually transitions<br>into the closed state, where switching to the open state is more probable.<br>Schematically these reactions may be written in a 2.5 T<br>A system<br>reactions<br>passing the lone<br>open stat<br>a refractor **2.5 Thermodynamic cycles**<br>A system contains a thermodynamic  $\alpha$  reactions and where starting from any<br>passing through a series of intermedia<br>Ion channels often undergo therm<br>open state, which allows ions to pass the are 2.5 Thermodynamic cycles<br>
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transitions<br>
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Schematically these reactions may be written in a circle. The ion channel is said to undergo<br>a thermodynamic cycle because the channel once open can go through the refractory and the<br>closed state before opening again. closed state before opening again. 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 obeys detailed balance then If we assume that each of these reactions is at equilibrium and obeys detailed balance then  $\frac{1}{2}$   $\frac{1}{2}$ 

<span id="page-10-0"></span>
$$
k_1C = k_{-1}O
$$
;  $k_2O = k_{-2}R$ ;  $k_3R = k_{-3}C$ . (2.41)

 $\alpha$  equations gives Rearranging these equations gives  $% \mathcal{N}$ Rearranging these equations gives

Rearranging these equations gives  
\n
$$
C = \frac{k_{-1}}{k_1}O = \frac{k_{-1}}{k_1} \cdot \frac{k_{-2}}{k_2}R = \frac{k_{-1}}{k_1} \cdot \frac{k_{-2}}{k_2} \cdot \frac{k_{-3}}{k_3}C
$$
\nand so equilibrium imposes a constraint on the reaction rates:

and so equilibrium imposes a constraint on the reaction rates:

<span id="page-10-1"></span>
$$
k_1k_2k_3 = k_{-1}k_{-2}k_{-3}.
$$
\n(2.43)

arbitrarily chosen and must obey Eq. 2.43, which implies that the probability of going round For a thermodynamic cycle that is<br>arbitrarily chosen and must obey E<br>the cycle one way is equal to the pro<br>If the rate constants do not satis<br>occur preferentially in one direction.<br>**Aside**<br>We can compute the times to go ar  $\frac{n-1}{2}n-3$ <br>reach equili<br>which implie<br>of going rou For a thermodynamic cycle that is able to reach equilibrium, the reaction rates may not be for not be g round<br>cycle to<br>nodeller.  $C =$ <br>and so equilibrium imposes a<br>For a thermodynamic cycle<br>arbitrarily chosen and must<br>the cycle one way is equal to<br>If the rate constants do n<br>occur preferentially in one div<br>**Aside**<br>We can compute the times to<br>rium.  $\begin{array}{ll} \mbox{\hbox{\small\it E-2}} & \mbox{\small\it E-2} \\ \mbox{\small\it F} & \mbox{\small\it V} & \mbox{\small\it C} \\ \mbox{\small\it D} & \mbox{\small\it F} & \mbox{\small\it W} \\ \mbox{\small\it R} & \mbox{\small\it C} & \mbox{\small\it E} & \mbox{\small\it E} & \mbox{\small\it C} \\ \mbox{\small\it R} & \mbox{\small\it E} & \mbox{\small\it E} & \mbox{\small\it E} & \mbox{\small\it C} & \mbox{\small\it E} & \mbox{\small\it E} & \mbox{\small\$ For a distributed procedure of a positive distribution and report of the cycle one way is equal of the rate constants occur preferentially in or **Aside** We can compute the tim rium.

 $\lim_{p \to \infty} A_p$ occur preferentially in one direction. A phenomenon that may not be intended by the modeller.<br>Aside the cycle one way is equal to the probability of going round the cycle the other way.<br>If the rate constants do not satisfy Eq. 2.43, the system is using energy to force t occur preferentiany in one direction. A phenomenon that may not be intended by the modeller.<br>Aside If the rate constants do not satisfy Eq. [2.43,](#page-10-1) the system is using energy to force the cycle to

#### Aside

Aside<br>We can compute the times to go around the cycle in each direction, which are equatium. equal a in molecular units (number of a substance per litre). Let  $\Gamma$ We can compute the times to go around the cycle in each directium.<br>
11 constants do not satisfy Eq. 2.43, the system is using energy to force the cycle to<br>ially in one direction. A phenomenon that may not be intended by the modeller.<br>te the times to go around the cycle in each direction, whi Found the system is using energy to force the cycle to<br>that may not be intended by the modeller.<br>each direction, which are equal at equilib-We can compute the times to go around the cycle in each direction, which are equal at equilib-<br>rium. rium.

Let the typical time for one clockwise transition around the cycle be  $T_{\text{C}}$  and so the typical rate for this cycle be  $1/T_C$ . We expect that the rate of a compound reaction, such as a clockwise transition, is approximately equal to the product of the probability of the most probable reaction and its rate [\[5\]](#page-63-4). The most probable clockwise transition is the direct cycle from, say, the closed to the open to the refractory and back to the closed state. Let this probability as  $P(c \rightarrow o \rightarrow r \rightarrow c)$ and its rate by  $1/t_d$ . Then we expect

<span id="page-11-3"></span>
$$
T_C^{-1} \simeq \mathcal{P}(\mathbf{c} \to \mathbf{o} \to \mathbf{r} \to \mathbf{c}) \times t_d^{-1}.
$$
 (2.44)

Intuitively, the typical clockwise transition time  $T_+$  is longer than the time  $t_d$  for a direct clockwise cycle because at least some anticlockwise reactions will also likely occur.

The probability  $P(c \to o \to r \to c)$  is a product of the probability of moving from the closed to the open state,  $\frac{k_1}{k_3+k_1}$ , and the probability of moving from the open to the refractory state,  $\mathbf{k}_2$  $\frac{k_2}{k_{-1}+k_2}$ , and the probability of moving from the refractory back to the closed state,  $\frac{k_3}{k_{-2}+k_3}$ :

<span id="page-11-1"></span>
$$
P(c \to o \to r \to o) = \frac{k_1}{k_{-3} + k_1} \times \frac{k_2}{k_{-1} + k_2} \times \frac{k_3}{k_{-2} + k_3}.
$$
 (2.45)

The rate of the direct clockwise cycle is the reciprocal of the total dwell time in the three states: closed, open, and refractory. Once the system enters a particular state, the dwell time is the average time spent there. It is determined by the number of reactions leaving the state. The dwell time for the closed state is  $\frac{1}{k_{-3}+k_1}$ ; for the open state it is  $\frac{1}{k_{-1}+k_2}$ ; and for the refractory state  $\frac{1}{k_{-2}+k_3}$ . The total dwell time is therefore

<span id="page-11-2"></span>
$$
t_d = \frac{1}{k_{-3} + k_1} + \frac{1}{k_{-1} + k_2} + \frac{1}{k_{-2} + k_3}.\tag{2.46}
$$

Using Eqs. [2.45](#page-11-1) and Eq. [2.46,](#page-11-2) we can find  $T_C$  from Eq. [2.44.](#page-11-3)

Similarly, if the typical time to transition anticlockwise around the cycle is  $T_A$  then we expect

$$
T_A^{-1} = P(c \to r \to o \to c) \times t_d^{-1}
$$
\n(2.47)

with

$$
P(c \to r \to o \to c) = \frac{k_{-3}}{k_{-3} + k_1} \times \frac{k_{-2}}{k_{-2} + k_3} \times \frac{k_{-1}}{k_{-1} + k_2}.
$$
 (2.48)

Consequently,

<span id="page-11-4"></span>
$$
\frac{T_C}{T_A} = \frac{P(c \to r \to o \to c)}{P(c \to o \to r \to o)}
$$

$$
= \frac{k_{-1}k_{-2}k_{-3}}{k_1k_2k_3}
$$

which gives Eq. [2.43](#page-10-1) when  $T_C = T_A$ .

#### <span id="page-11-0"></span>2.6 Ultrasensitivity and the Hill number

The response curve of system is its input-output relationship and gives the steady-state level of output as a function of the level of input. Many empirical response curves may be approximately described by a Hill function.

because only one molecule of dimer $\frac{1}{\sqrt{2}}$  $\frac{1}{25}$   $M$  membrane receptors receptors receptors receptors receptors receptors receptor-receptor-receptor dimerize to  $\frac{1}{2}$ because only one molecule of dimer  $\overrightarrow{G}$  in  $\overrightarrow{G}$  is  $\overrightarrow{G}$  is unumi because only one molecule of dimer  $\frac{\delta}{\sqrt{n}} \log \frac{\delta}{\sqrt{n}}$  diates. Summing let  $\sum_{n=1}^{\infty} \frac{\delta}{\epsilon} \log \frac{1}{\epsilon} \log \frac{\delta}{\epsilon}$  and  $\frac{\delta}{\epsilon} \log \frac{1}{\epsilon} \log \frac{1}{\epsilon}$  and  $\frac{\delta}{\epsilon} \log \frac{1}{\epsilon} \log \frac{1}{\epsilon}$  of time dt the association and because only one molecule of dimer  $\frac{1}{\sqrt{2\pi}}$  set  $\frac{1}{\sqrt{2\pi}}$  are  $\frac{1}{2}$ . Summing 1<br>  $\frac{1}{2}$   $\frac{1}{$ because only one molecule of dimer  $\frac{1}{\sqrt{6}}$  reaction and  $\frac{1}{\sqrt{6}}$  reaction and  $\frac{1}{\sqrt{6}}$  reaction and  $\frac{1}{\sqrt{6}}$  reaction and  $\frac{1}{\sqrt{6}}$  reaction. Although the association released  $\frac{1}{\sqrt{6}}$  reaction. Al beca<br>
25  $\tilde{f}$ <br>
25 inter<br>
at the rate for the rate<br>
inter<br>
different<br>
time<br>
react<br>
Taki<br>
hc because only one molecule of dimer  $\sum_{k}$   $\sum_{k}$  and  $\sum_{k}$  summing i<br>  $\sum_{k}$   $\sum_{k}$   $\sum_{k}$   $\sum_{k}$  and  $\sum_{k}$  the association and  $\delta$  such reactions. We can<br>
index are involved in the association and  $\delta$  sociati because only one molecule of dimer  $\frac{66}{\sqrt{2}}$  is  $\frac{66}{\sqrt{2}}$  in  $\frac{1}{\sqrt{2}}$  Summing 1 because only one molecule of dimer **interaction**  $\mathbf{g}^T$  or dissociates. Summing Eq. 3 and the interaction of dissociation reading the consistents in the societies of a state of and twice Eq. 9 and twice Eq. 9 and twic  $A$  and  $B$ <br>mber of  $\lim_{a \to a} E$ e ∤u<br>nd m d<br>Barne  $\frac{dA}{dR}$ <br>  $\frac{dR}{dR}$  = constant =  $N_C$ , change<br>  $\frac{dR}{dR}$  in reactions will b<br>  $A$  and  $B$  molecules i<br>  $\frac{dR}{dt}$  =  $\frac{dR}{dt}$   $\frac{dR}{dt}$  is the<br>  $\frac{dR}{dt}$  is the<br>  $\frac{dR}{dt}$  is the<br>  $\frac{dR}{dt}$   $\frac{dR}{dt}$   $\frac{dR}{dt$ because only one mass of the different of the different of the different of  $\frac{R}{16}$  is the concentration of  $\frac{R}{16}$  and  $\frac{R}{16}$  and  $\frac{R}{16}$  and  $\frac{R}{16}$  and  $\frac{R}{16}$  is the concentration of  $\frac{R}{16}$  is t because only one molecules<br>  $25$  **Aregulary** (describe line at the signal properties)<br>
increased of time at the signal associate<br>  $\frac{\partial P}{\partial x}$  in an alternative, the multiple state,<br>  $\frac{\partial P}{\partial x}$  in an alternative concent because only one molecule of dimer  $\frac{d}{dt}$  is  $\frac{d}{dt}$  and  $\frac{d}{dt}$ <br>  $\frac{d}{dt}$   $\frac{d}{dt}$   $\frac{d}{dt}$  (hence it are association and  $\frac{d}{dt}$  is score it and  $t$ <br>  $\frac{d}{dt}$   $\frac{d}{dt}$   $\frac{d}{dt}$   $\frac{d}{dt}$  are  $\frac{d}{dt}$  and because only one molecule of dimer  $\frac{1}{16}$   $\frac{1}{16}$ molecule of dimer**ical interaction**<br>  $u_{\text{new}}$  is  $\overline{\mathcal{C}}$  and  $\overline{\mathcal{C}}$  constant interactions will be<br>
the association and dissociation reactions will be<br>
later). The number of pairs of A and B molecules is<br>
ssociat because only one molecule of dimer<br>
Many states. Summing i<br>
Many membrane receptors how the number<br>
of the dimerical of the association and dissociation reactions will be<br>
a receptor-receptor-receptor-receptor-receptor-re because only one molecule of dimer  $\frac{1}{k}$   $\frac{1}{$ because only one molecule of dimer  $\frac{1}{\sqrt{2}}$  section  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$ . Change interval of time dt, the association and dissociation reactions because only one molecule of dimer  $\frac{\sigma_f^2}{\sigma_f^2}$  is  $\frac{\sigma_{\text{start}}}{\sigma_f^2}$  are  $\frac{\sigma_{\text{start}}}{\sigma_f^2}$  are removed with  $\frac{\sigma_{\text{start}}}{\sigma_f^2}$ because only one molecule of dimer  $\frac{G_F^2}{\sqrt{g}}$  is  $\frac{G_F^2}{\sqrt{g}}$  and  $\frac{G_F^2}{\sqrt{g}}$ . Summing l reaction proceeds at the rate <sup>f</sup> <sup>R</sup>[  $\mathbb{E}[\mathbb{R}] + \mathbb{E}[\mathbb{R}_2] = \text{constant} \equiv 2[\mathbb{R}]_0 + 2[\mathbb{R}_2]_0$ <br>umber of C molecules at a time  $t + dt$  is the while mumber of pairs of  $A_0$  and B molecules<br>e over  $R_1^*$  time  $R_2^*$ . The number of  $C$  molecule<br>example  $R_1^*$  that  $\frac{1}{C}R_2^*$  is constant  $\frac{1}{C}R_1^*$   $\frac{1}{C}R_2^*$  is constant.  $\frac{d}{dx} + \frac{d}{dx} \frac$ time t plus the number gained in association reactions and minus the interestions.<br>
The off dimerical terms  $\left\{\begin{array}{l}\sum_{k=1}^{k-2} \text{diag}\left\{\text{diag}\right\}\right\} \text{diag}\left\{\text{diag}\right\}\right\}$ <br>
Taking the limit of dt going to a weapon and the num  $\lim_{t\to\infty} \frac{\partial^2 u}{\partial t} = \lim_{t\to\infty} \frac{\$ **S fareformal** of time dt, the association and dissociation reactions will b scales in the value of the association and dissociation reactions will be scaled by  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{$  $\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)$  $\text{tr}\left[\text{colimers}\right] \text{dim}\left[\text{colimers}\right]$ time t plus the number gained in association reactions and minus the interestions.<br>
We off dimerment of the property of the state of  $\frac{\sum_{k=2}^{k-2} \sum_{k=1}^{k-1} \prod_{k=1}^{k} \sum_{k=1}^{k} \sum_{k=1}^{k} \sum_{k=1}^{k} \sum_{k=1}^{k} \sum_{k=1}^{k} \$  $350$  cases called the and  $\alpha$  and  $\alpha$  and  $\alpha$  and  $\alpha$  response  $\alpha$  in the  $\alpha$ with a mumber of pairs of data in  $H$  molecules  $\frac{1}{2}$  we have the set of  $\frac{1}{2}$  $\sim$   $\cdot$  $\frac{1}{2}$   $\frac{1}{2}$  bative. Therefore, the number of C molecules at a time  $t + dt$  is the<br>time t plus the number gained in association reactions and minus the i Taking the limit of dt going to zero, we dissociate<br>how the number of  $C$  moderation  $N_C$ , change<br>association and **dissociation**  $\tilde{P}$  redetions will b because only one molecule of dimer to use  $\frac{1}{\sqrt{2}}$   $\frac{1}{2}$   $\frac{1}{$  $\text{because}$ <br> $\text{Area}$ because only one molecule of dimer<br>  $\sum_{\text{interval}} \mathbf{S} \mathbf{R} \mathbf{U} \mathbf{S} \mathbf{R} \mathbf{U}$  and time dt, the association<br>  $\sum_{\text{refall}} \mathbf{S} \mathbf{U} \mathbf{R} \mathbf{U}$  are uncheanously in the matrix<br>  $\sum_{\text{refall}} \mathbf{V} \mathbf{U} \mathbf{S} \mathbf{V}$  are because only one molecule of dimer **lanet of**  $\mathcal{B}$  considerables,  $\mathcal{N}_{\text{c}}$  increases in the solution and dissociation receptors respectively of time  $d$ , the association receptor-receptor-receptor-receptor-recept because only one molecule of dimer **and**  $\frac{d}{dt}$ ,  $\frac{$ because only one molecule of dimer  $\frac{\sum_{i=1}^{\infty} \sum_{i=1}^{\infty} \sum_{$ because only one molecule of dimer $\frac{\sum_{i=1}^{\infty} \frac{1}{2} \sum_{i=1}^{\infty} \$ because and sue andered of dimer $\frac{\sum_{i=1}^{\infty} \frac{1}{2} \sum_{i=1}^{\infty} \frac{$ ause only one molecule of dimer $\frac{1}{\sqrt{2}}$  is  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  are discontributed by  $C$ , we also claim and  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  and  $B$  concerned b  $\frac{d}{dx}$ 2or this second is the set of  $R_2^r$  ( $R_2^r$  or  $R_2^r$ ) and  $R_2^r$ ) = constant  $\equiv$   $R_2^r$  ( $R_2^r$ ) = constant  $\equiv$   $R_2^r$  ( $R_2^r$  molecules in the because only one molecule of dimer $\frac{1}{2}$ s denotes are involved in the control of the control of the control of the dimerricules are interesting in the dimerricules are interesting in the dimerricules are interesting in  $\begin{array}{r}\n\text{per} \\
\equiv\n\end{array}$ Example 1<br>  $N_C$ , change<br>
tions will b<br>
i molecules i<br>  $\begin{array}{l} \nC \text{ model} \ C \ \uparrow \text{cell} \ \uparrow \text{cell} \ \uparrow \text{cell} \ \uparrow \text{cell} \ \uparrow \text{final} \ \uparrow \text{final} \ \uparrow \text{final} \ \uparrow \text{final} \ \uparrow \text{real} \ \uparrow \$ because only one molecule of dimer **and**  $\phi$  and  $\phi$  is expected.<br>
before,  $\phi$  and twice only one contribute of the contribution and forms or dissociates.<br> **Equipmentation because of the contribution** of the summing eq where  $\frac{A}{\sqrt{2}}$  is the concentration of the concentration of  $\frac{B}{\sqrt{2}}$  is the mumb if  $\frac{B}{\sqrt{2}}$  is the mumb if  $\frac{B}{\sqrt{2}}$  is the mumb of  $\frac{B}{\sqrt{2}}$  is the mumb of  $B$  and  $\frac{B}{\sqrt{2}}$  is the mumb of  $\frac{B}{\sqrt{$ the<br>same only one molecule of dime  $\frac{\sqrt{10}}{\sqrt{10}}$ ,  $\frac{60}{20}$ ,  $\frac{1}{20}$ ,<br>  $\frac{1}{20}$ ,  $\frac{1}{20}$ because only one molecule of dimer $\frac{1}{\sqrt{2}}$  of  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  and because only one molecule for those the dimer $\frac{1}{\sqrt{2}}$  on  $\frac{d\bar{x}}{dx}$  on  $\frac{d\bar$ because only one molecules to times  $\frac{\sum_{i} \phi_{i}^{(k)} \equiv \sum_{i} A_{i}^{(k)}$ . Summing 1<br>
28 denote a right of receptor and disconsistent and disconsistent in the reaction of dimer of reaction and disconsistent in the reaction of because only one molecule of dimer  $\frac{1}{\sum_{i=1}^{n} \sum_{j=1}^{n} \sum_{j=1}^{n}$ because only one molecule of dimer $\frac{\sum_{i=1}^{\infty} \frac{1}{2} \sum_{i=1}^{\infty} \$ reactives it is the controller of the rate because only one molecule of dimer of  $\overline{G}$  and  $\overline{G}$   $\overline{G}$  and  $\overline{G}$   $\overline$ because only one molecule of dimer  $\frac{\partial f}{\partial x}$  and  $\frac{\partial f}{\partial y}$  and  $\frac{f}{\partial z}$ . Summing l only one molecule of dimer<br>  $\frac{1}{\sqrt{2}}$  is  $\frac{1}{\sqrt{2}}$  in the association and dissociation because only one molecule of dimer **forms or**  $\mathbb{Q}$  with  $\mathbb{Q}$  is  $\mathbb{Q}$  is  $\mathbb{Q}$ . Summing Eq. 8 and  $\mathbb{Q}$  is  $\mathbb{Q}$  is  $\mathbb{Q}$  is  $\mathbb{Q}$  is  $\mathbb{Q}$  is  $\mathbb{Q}$  is  $\mathbb{Q}$ . Eq. 9 and  $\mathbb{Q}$  is the d now the number  $\omega_1 \leftarrow \frac{1}{2} \frac{1}{N_C} \equiv 0^{IV_C}$ , changes association and  $\frac{1}{N} \frac{1}{N_C} \approx 0^{IV_C}$ .  $\frac{\text{refactor}}{\text{refactor}} \text{arg}(t) + \frac{\text{ref}}{\text{ref}}(t) + \frac{\text{ref$ time t plus the number gained in association reactions and minus the interest of the number gained in association reactions and minus the interest of  $\frac{d}{dx}$ riying that, and the set  $\Gamma$  a number of pairs of  $A$  and  $B$  molecules which is experimented of going round  $\frac{1}{2}$  and  $\frac{1}{2}$  of  $\frac{1}{2}$  in  $\frac{1}{2}$ number of C molecules at a time  $t + dt$  is fl  $\frac{1}{2}$  he nun  $\epsilon$ because only one molecule of dimer  $\frac{\overbrace{\text{of}}_{\text{other}}}{\overbrace{\text{of}}_{\text{other}}}$  of  $\overbrace{\text{of}}_{\text{other}}$   $\overbrace{\text{of}}_{\text{other}}$   $\overbrace{\text{of}}_{\text{other}}$   $\overbrace{\text{of}}_{\text{other}}$   $\overbrace{\text{of}}_{\text{other}}$  and  $\overbrace{\text{of}}_{\text{other}}$   $\overbrace{\text{of}}_{\text{other}}$   $\overbrace{\text{of}}_{\text{other}}$   $\overbrace{\text{of}}$ because only one molecule of dimer $\frac{1}{\sqrt{2}}$  solutions  $\frac{1}{2}$ . Summing 1<br>  $\frac{1}{2}$   $\$ because only one molecule of dimer **can be dimerified** and signal downstream.<br>
So  $\widehat{A}U$  and  $\widehat{A}U$  and  $\widehat{B}U$  and because only one molecule of dimer  $\frac{1}{\log_2 R}$  of  $\frac{1}{\log_2 R}$ . Summing <br>  $\frac{1}{2}$   $\frac{$ because only one molecule of dimerent  $\mathbf{R}$  are  $\mathbf{R}$  are removed in the removed in  $\mathbf{R}$  are removed in the removed in the removed in the removed in the system are removed in the system and  $\mathbf{R}$  are removed because only one molecule of dimer $\blacksquare$ <br>
See Apple interest in a membed for  $c$  polificial<br> $\Delta p_{\rm ex}$ , then  $\beta$  released by the breaction and the<br>second by the breaction and the properties are released by the breaction. **Example 12**<br>
interval of time dt, the final of time dt, the range is concluded in the range of the rate of the number of  $\epsilon$  over  $R$  time the numb blecule of dimer  $\frac{\frac{1}{\sqrt{2}}\sum_{k=1}^{N} \sum_{k=1}^{N} \sum_{k=1}^{K} \sum_{k=1}^{N} \$ of pair<br> $d_t^t$ . The set of  $\mathbb{F}_2^{d_t}$ because only one molecule of dimer **Election Material** space is summing in the dimerry of the later the manned of particular No. denotes a substitutions. The annihology conditions are interesting in the dimerry of the dim d[R2]  $\begin{aligned} \n\begin{bmatrix}\n\mathbf{i} \\
\mathbf{j}\n\end{bmatrix} \\
\mathbf{j}\n\end{aligned}$ because only one molecule of dimer **Exerc**ise Summing :<br>
Summing is equally dissociate for the numerical of case of a sum diversion and the sum dissociates. The anniver of pairs of and twice Eq. 9. along the summing pairs because only or<br>  $\frac{1}{2}$  into an equation of the rate of change of the rate of change<br>  $\frac{1}{2}$  into  $\frac{1}{2}$  in because only one molecule of time  $\frac{1}{\frac{1}{2}}$ <br>  $\frac{1}{25}$  **SUPARMON** accrite two the annual of  $\frac{1}{25}$ <br>  $\frac$ Theirs the finit of decomposition<br>and decomposition and descentrations in the number of  $\frac{d\cos\alpha}{dx}$ <br>association and dissociations  $\frac{d\cos\alpha}{dx}$ .<br>when anywher of pairs of odd and<br>e over  $\frac{d\cos\alpha}{dx}$  impediate  $\frac{d\cos\alpha}{$ dt <sup>=</sup> <sup>f</sup> <sup>A</sup>[ ][<sup>B</sup> The units of the main of the matter of the maximal state is the properties of the matter of the beca<br>s 15 ft<br>inter<br>s 62 because only one molecule of dimer $\frac{\sum_{i=1}^{\infty} \frac{1}{2} \sum_{i=1}^{\infty} \$  $H_{21}^{th} = \text{length of } G_{\text{max}}$ <br>
notecules at a time  $t + dt$  is the<br>
notecules at a time  $t + dt$  is the<br>
ciation reactions and minus the is<br>  $\frac{1}{t + 1}$ <br>  $\frac{1}{t$ Many membrane receptors receptors receptors receptors relations of the symphonic polynomial of the sometimes receptor of the form and some control of the sometimes of the sometimes of the sometimes of the sometimes of the because only one molecule of dimer  $\frac{1}{\sqrt{2}}$   $\frac{$ **Let R denote a receptor and R2** denotes satisfy the reaction and  $\mathbf{Z} \in \mathbb{R}$  denotes the reaction of reaction and R2 denotes a reaction  $\mathbf{Z} \in \mathbb{R}$  denotes the reaction of reaction  $\mathbf{Z} \in \mathbb{R}$  denotes sati because only one molecule of dimer<br>  $\frac{1}{16}$  is  $\frac{1}{16}$  in  $\frac$ because only one molecule of dimer  $\frac{f_{\text{max}}}{\sqrt{6\pi}}$  reactions. Summing 1<br>  $\frac{1}{25}$  **AFF** $\frac{1}{25}$  **AFF** $\frac{1}{25}$  **C** reaction and  $\frac{1}{25}$  **C**  $\frac{1}{25}$  released by the association and  $\frac{1}{25}$  **C** and  $\frac{1}{$ because two R molecules are involved in both reactions. The dimer, R , obeys 2 association and **di**ssoci<del>atida</del>l redetions will b<br>with a mumber of mairs of day and B molecules<br>e over a time dt. The number of C molecule  $N_C(\mathbf{k}_2 + d\mathbf{l}) = \mathbf{R}_C(\mathbf{l}) + \mathbf{j} d\mathbf{l} N_A N_B - \text{ballN} g\mathbf{l}$ <br>Taking the limit of dt going to a referable zero, we have these pairs will associate over a time  $dt$ . The number of C intended by the modeller of  $C$  in  $\frac{1}{2}R_0 + d$ ,  $\frac{1}{2}d$  in  $\frac{1}{2}d$ ,  $\frac{1}{2}d$ , 2 Ultrasensitivity, cooperativity, and Hill numbers e over  $R_1$  time  $dt$ . The number of C molecule<br>umber of C molecules at a time  $t + dt$  is the<br>umber of C molecules at a time  $t + dt$  is the<br>If the output, *u*, increases with increasing levels of input, *x*, the appropriate H because only one molecule of dimer  $\frac{\partial \hat{\mathbf{f}}^*}{\partial \hat{\mathbf{f}}^*} \text{ns} \overline{\mathbf{\hat{f}}^*_{\mathbf{g}}}$   $\frac{\partial \hat{\mathbf{f}}}{\partial \mathbf{g}} \overline{\mathbf{\hat{f}}^*}$ . Summing l  $\frac{f_1}{f_1}$ ns $\frac{f_2-2}{f_2}$ istagia)  $\frac{\sum_{i=1}^{k_2} \sum_{j=1}^{k_1} \sum_{j=1}^{k_2} \sum_{j=1}^{k_2} \sum_{j=1}^{k_1} \sum_{j=1}^{k_2} \sum_{j=1}^{k_2} \sum_{j=1}^{k_1} \sum_{j=1}^{k_2} \sum_{j=1}^{k_2} \sum_{j=1}^{k_2} \sum_{j=1}^{k_1} \sum_{j=1}^{k_2} \sum_{j=1}^{k_2} \sum_{j=1}^{k_2} \sum_{j=1}^{k_2} \sum_{j=1}^{k_2} \sum_{j=1}^{k_2} \sum$  $\widehat{C}^2$   $\sum_{k=1}^{n}$   $\sum_{k=1}^{n}$  (8)  $\sum_{k=1}^{n$ r). The<br>iate over The number of pairs of A and B molecules i<br>te over  $R_1^{\text{th}}$  time  $R_2^{\text{th}}$ . The number of C molecules<br>number of C molecules at a time  $t^0 + dt^0$  is the<br>gained in association reactions and minus the i because only one molec<br>  $\frac{X}{Y}$   $\frac{X}{Y}$   $\frac{X}{Y}$   $\frac{X}{Y}$   $\frac{X}{Y}$   $\frac{X}{Y}$  and  $\frac{X}{Y}$  the concentration of time dt, the<br>  $\frac{X}{Y}$   $\frac{X}{Y}$   $\frac{X}{Y}$   $\frac{X}{Y}$  and  $\frac{X}{Y}$  and  $\frac{X}{Y}$  and  $\frac{X}{Y}$  are def because only one molecule of dimer  $\frac{1}{\sqrt{2}}$  is different and dissociation reading  $C$  with  $\frac{1}{2}$  and  $\frac{1}{2}$  are specific reading and are second and are simplified. The manner of pairs of A and B n  $\frac{1}{2}$  are meaning one molecule of dimerimerize to monoids of  $C$  and<br> $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$ <br> $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  an because only one molecule of three **can bind ligand and signal downstream**. The dimerization reaction reaction reaction is unusual.<br>  $\frac{1}{2} \sum_{n=1}^{\infty} \frac{\partial^2 n}{\partial n} \frac{\partial^2 n}{$ because only one molecule of dimer **LEt of Additional** Summing <br>
S **Area** related by the sected on and additional content on the reaction of receptors. No since  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{$ d in ass<br> $\frac{K-2}{2}$ Exercise only are nodes<br>be dimensionless for the massive of galaxies, Archeon computer is suppressed to the<br>system are removed for the massive of R are removed to the system are<br> $\frac{1}{16}$ . The removed molecules computer by the first properties are the first properties are released by the association and denoted by the associatio blecule of dimer **lables**  $\frac{1}{2}$   $\frac{1$  + 2b[R2] (8) Because the multiple of the second of the second interests are involved in the dimer,  $R_{\text{min}}$  and  $R_{\text{min}}$  are involved in the dimerricules are involved in the dimerricules are involved in the dimerricules are in the d  $\frac{1}{3}$   $\frac{1}{3}$  Notice cally one molecule of the macroscopic function of the matrix  $\sum_{k=1}^{\infty} \frac{1}{k}$  and  $\sum_{k=1}^{\infty} \frac{1}{k}$  a From each year match is contained to form a receptor of the simulation of the because only the molecule of dimer **Can** bind  $\hat{R}$  can be signal downstream.<br> **on Angle Control** can be signal down to denote the signal model of the dimerical can be a signal down of density and the dimerical properti because only as moderate at more models of denote a reaction of the reaction of  $\frac{y(x)}{y_{\text{max}}} = \frac{x^2}{n^2 + x^2}$ .<br>
Let  $\frac{y(x)}$ because only one molecules of these **molecules**  $\frac{1}{2}$  is system and the rate rate of the rate conductions of  $\frac{1}{2}$  is a system of the rate of the removement of  $\frac{1}{2}$  are  $\frac{1}{2}$  and  $\frac{1}{2}$  are removed in because only one molecule of those **and the control of the control of the release of reaction** and the secondary of the molecules are released by the property of the association and the secondary of the association. Altho because only one molecule of dimer  $\frac{dR}{dt}$   $\frac{dR}{dt}$  and  $\frac{dR}{dt}$ be of dimer **and reaction**  $\mathbf{K}^{\text{obs}}$  and  $\mathbf{K}^{\text{obs}}$  are involved in an detection and described in excitions. Will be number of pairs of A and B molecules in the dimer,  $\mathbf{K}^{\text{obs}}$  and  $\mathbf{K}^{\text{obs}}$  and  $\mathbf{K$ molecule of dimer **forms of dissociation**<br>secribe how the number of pairs of  $A$  and  $B$  molecules is<br>the the secondition and dissociated reactions will be later). The number of pairs of  $A$  and  $B$  molecules is<br>sociate o  $\frac{1}{2}$  members of  $\sigma$  morecules as a since  $v + \omega$  receptor-receptore over  $R_1^*$  time  $R_2^*$ . The number of  $C$  molecule association and  $\overline{db}$  is  $+\overline{d}R\overline{c}$   $\overline{d}$   $\overline{d$  $\overline{\text{W}}$ ง<br>ท  $\frac{1}{2}$ Taking the limit of dt going to zero, we have<br>
now the number of C name of the contract of the division of the divisory of the contract of the contract of the same of the contract of the same of the contract of the contra  $\cdots$  over  $R_1$   $\downarrow$   $\cdots$   $R_2$ . k2  $\frac{1}{2}$  $\frac{1}{b}$  ( $\frac{1}{b}$   $\frac{1}{b}$   $\frac{1}{b}$   $\frac{1}{b}$   $\frac{1}{b}$   $\frac{1}{c}$   $\frac{1}{c}$   $\frac{1}{c}$   $\frac{1}{c}$   $\frac{1}{d}$   $\frac{1}{c}$   $\frac{1}{d}$   $er$  $\frac{1}{2}$ only the dimer can bind ligand and signal downstream. The dimerization is unusual. The dimerization reaction is unusual. because only one molecule of dimer  $\frac{\overbrace{\mathbf{C}}_{\mathbf{R} \text{max}}^{\mathbf{C}}}{\mathbf{C}}$  denotes. Summing l because only one molecule of dimer **of the Case of Articular**<br>  $\mathbf{F}$ **S**  $\mathbf{F}$ **C**  $\mathbf{F}$   $\mathbf{F}$ because only one molecule of dimer  $\frac{1}{\sqrt{6}}$  is  $\frac{1}{\sqrt{6}}$  and  $\frac{1}{\sqrt{6}}$ . Summing 1<br>  $\frac{1}{25}$   $\frac{1}{\sqrt{6}}$   $\frac{1}{\sqrt{6}}$   $\frac{1}{\sqrt{6}}$   $\frac{1}{\sqrt{6}}$   $\frac{1}{\sqrt{6}}$   $\frac{1}{\sqrt{6}}$   $\frac{1}{\sqrt{6}}$   $\frac{1}{\sqrt{6}}$   $\frac{1}{\sqrt{6}}$  because only one molecule of dimer **of**<br>  $\mathbf{F}$ <br>  $\mathbf{F}$ <br> because the number of dimer  $\frac{L}{d\theta}$  or  $\frac{L}{d\theta}$  and  $\frac{L}{d\theta}$  and  $\frac{L}{d\theta}$ . Summing in  $\frac{L}{d\theta}$  and  $\frac{L}{d\theta}$  and  $\frac{L}{d\theta}$  c  $\frac{L}{d\theta}$  with reactions will be the three involved in a second in an diss ule of dimer  $\overbrace{\text{L}_\text{B}\otimes\overleftrightarrow{q_\text{min}}\text{ln}[\underline{L}_\text{M}]}\text{a}_\text{G}c_\text{S}$ . Summing 1<br>has concident and *dissociation* and  $\overline{q_\text{min}}$ . The number of pairs of *A* and *B* molecules is<br> $\overline{e}$  over  $\overline{p_\text{B}}$  time  $p_\text{eff}$ because on<br>
S.  $\tilde{A}^{\text{tr}}$  is  $\tilde{a}^{\text{tr}}$  in the red of<br>
interval of  $\tilde{b}^{\text{tr}}$  is  $\tilde{b}^{\text{tr}}$  in  $\tilde{b}^{\text{tr}}$  is  $\tilde{b}^{\text{tr}}$  in terms terms of  $\tilde{b}^{\text{tr}}$  in terms of  $\tilde{b}^{\text{tr}}$  is  $\tilde{b}^{\text{tr}}$ because only one <br>  $\frac{1}{2}$  in  $\frac{1}{2}$  is  $\frac{1}{2}$  in the denoted of three denotes of the model<br>  $\frac{1}{2}$  is  $\frac{1}{2}$  in  $\frac{1}{2}$  in  $\frac{1}{2}$  in  $\frac{1}{2}$  in  $\frac{1}{2}$  in  $\frac{1}{2}$  in the model of a substance the is Avogadro's number and V is the volume of the cell in the cell i because only one molecule of dimension is a space of the dimensional different of the concentration for the number of the munder of  $\frac{1}{\sqrt{2}}$ . Therefore, the number of the concentration of  $\frac{1}{\sqrt{2}}$ . The must divide withe mumber of pairs of<br>
e over  $R_1^3$  time  $R_2^d$ . The m<br>
umber of C molecules a<br>
neceases with increasing level<br>  $\frac{y(x)}{y_{\text{max}}} = \frac{y(x)}{K^r}$ <br>
Hill number, or occasionally t  $\begin{array}{r} \text{620G} \ \text{imply} \ \text{phog} \ \text{5dFN}_C \ \text{time } t \end{array}$ <br>These  $\begin{array}{r} \text{5dFN}_C \ \text{time } t \end{array}$ because only one molecule of dimer $\frac{1}{\|B\|^{2}}$  is the concentration of A and finite dimerrike of the concentration of A and finite dimerrikant properties of  $\frac{1}{\|B\|}\frac{1}{\|B\|}\frac{1}{\|B\|}\frac{1}{\|B\|}\frac{1}{\|B\|}\frac{1}{\|B\|$ because only one molecule of dimer  $f$  reactions  $\mathbb{R}$ <br>  $\mathbb{R}$   $\mathbb{R}$   $\mathbb{R}$   $\mathbb{R}$   $\mathbb{R}$   $\mathbb{R}$   $\mathbb{R}$   $\mathbb{R}$   $\mathbb{R}$  interval of time dt, the association and dissociation results of the molecule of because only one molecule of dimer  $\frac{\frac{1}{\left(\frac{1}{\sqrt{1-\lambda}}\right)}\sum_{k=1}^{n} \frac{1}{\lambda}}{\sum_{k=1}^{n} \frac{1}{\lambda}}$ . Summing 1<br>  $\sum_{k=1}^{n} \frac{1}{\lambda} \sum_{k=1}^{n} \frac{1}{\lambda} \sum_{k=1}^{n} \frac{1}{\lambda} \sum_{k=1}^{n} \frac{1}{\lambda} \sum_{k=1}^{n} \frac{1}{\lambda} \sum_{k=1}^{n} \frac{1}{\lambda} \sum$ because only one molecule of dimer  $\frac{\text{for } \text{first} \text{ distinct}}{\text{interior } \text{ different}}$ because only one molecule of dimer  $\frac{1}{(d+\beta)^2}$  s  $\frac{1}{(d+\beta)^2}$  and  $\beta$ . Summing 1<br>
28  $\frac{1}{(d+\beta)^2}$  if the *dt*, the association and  $\frac{d}{d+\beta}$  conclusions will be<br>
shown interval of time *dt*, the association and because only one molecule of dimer  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$   $\frac{1}{2}$ <br>  $\frac{1}{2}$ <br> because only one molecule of dimer  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$ . Summing 1<br>  $\therefore$   $\overrightarrow{AB}$  or  $\overrightarrow{AB}$  of time *dt*, the association and *dissociation* reactions will be<br>
such that is the *dt*, the associ because only one molecule of dimer  $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$ . Summing 1<br>  $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$  of time *dt*, because only one molecule of dimer of  $\mathbb{R}^2 \subset \mathbb{R}^d$   $\mathbb{R}^2$  denotes. Summing 1<br>
25  $\mathbb{R}^2$  **denotes a** reaction and  $\mathbb{R}^2 \subset \mathbb{R}^d$   $\mathbb{R}^d$  and  $\mathbb{R}^d$  of time *dt*, the association and  $\mathbb{R}^d$   $T$  $\mathbf{b}^{\mathcal{L}}$  ${}^{2}$ [K]<sub>0</sub> + 2[K<sub>2</sub>]<sub>0</sub><br>ne *t* + *dt* is the  $y_1^{\iota} =$ the photon can all associate over  $R_1$  ime<sub>r</sub> $dt$ . The number of C molecule:<br> $ddN_C$ . Therefore, the number of C molecules at a time  $t + dt$  is the  $\frac{1}{C\left(\frac{k_2}{\sigma}\right)}$  and  $\frac{1}{C\left(\frac{k_2}{\sigma}\right)}$  or  $\frac{1}{C\left(\frac{k_2}{\sigma}\right)}$  or  $\frac{1}{C\left(\frac{k_2}{\sigma}\right)}$  $\lim_{M \to \infty} \frac{\log \log \log M}{\log \log \log \log M}$   $N_C$ , chang because only one molecule of dimer  $\frac{G_F}{G}$  as  $\frac{G_F}{G}$  and  $\frac{G_F}{G}$  and  $\frac{G_F}{G}$  are interval of time dt, the association and dissociation reactions will see the matrix of the matrix of the matrix of the matrix o because only one molecule of dimer  $\frac{1}{\sqrt{2}}$  in  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  are units of time dt, the association and dissociation reactions  $\frac{1}{\sqrt{2}}$  are  $\frac{1}{\sqrt{2}}$  are  $\frac{1}{\$ becaus<br>
25 **Jan**<br>
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hov because only one molecule of dimer **Last**  $\frac{d}{dt}$  is  $\frac{d}{dt}$  and sometimes in  $\frac{d}{dt}$  and  $\frac{d}{dt}$  and because only one molecule of dimer  $\frac{1}{\sqrt{2}}$  is  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  are  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  of the dimerical of the dimerical and signal downstream. The intermediate vertices in the because only one molecule of dimer  $\mathcal{L}(\mathcal{L})$  denotes. Summing is<br>  $\mathcal{L}(\mathcal{L})$  and  $\mathcal{L}(\mathcal{L})$  an limer <mark>fo<del>f i</del>ns <del>Con</del>dits tod</mark>iates.<br>In a number of Right of the production because only one molecule of dimer **are relatively defining** and B are interval of time  $dt$ , the association and **dissociation** reactions will be attacked later). The unumber of pairs of A and B molecules  $\frac{1}{2}R\frac{d}{dt}$ because only one molecule of dimer  $\frac{1}{\sqrt{2}}$  is  $\frac{1}{2}$   $\frac{1}{2}$  Taking the limit of *dt* going to zero, whow the number of  $\mathcal{L}$  association and **dissociation**<br>association and **dissociation**<br>which a number of pairs of<br>e over  $R$  time  $R$  imperiments and<br>umber of  $C$  molecules a<br>umb !t order and the discociation reaction reaction  $\frac{d}{dt}$   $\frac{d}{dt}$   $\frac{d}{dt}$   $\frac{d}{dt}$   $\frac{d}{dt}$  reactions will be of pairs of A and B molecules if  $R^d$ . The number of  $C$  molecules if  $R^d$ . The number of  $C$  molecules at  $\frac{\partial}{\partial \theta}$ <br> $\frac{\partial}{\partial \theta}$  $\mathbf{e}\mathbf{s}_t$ ming ]<br>change<br>will b<br>cules i<br>algeule because of dimer  $\frac{1}{\sqrt{2}}$  molecules. Summing is  $\frac{1}{2}$  and  $\frac{g}{h}$ because of dimer **hasten of controlled** conditions in the molecule of dimerical conditions of a and B molecule is the condition of particle of dimerical conditions of dimerical conditions and twice  $\mathbf{f}$  and  $\mathbf{f}$  a convert Eq. 3 into an equation for the rate of  $\frac{dx}{dx}$ . The rate of  $\frac{dx}{dx}$  into  $\frac{dx}{dx}$  association and  $\frac{dx}{dx}$  association and  $\frac{dx}{dx}$  association and  $\frac{dx}{dx}$  because only one molecule of dimer **Langestrich**<br>
So  $\widehat{H}_{\text{H}}^{\text{reg}}$  is the unit sum bundel  $\widehat{H}_{\text{G}}^{\text{reg}}$ <br>
index<br>
index<br> with a number of pairs of Anand B molecule<br>
e over  $R$  time  $R$  = constant =  $R$  C molecule<br>
umber of C molecules at a time  $t + dt$  is t where are interesting to reaction  $D$  more<br>cover  $R$   $\pm 2R_2$   $\pm 2$  constant  $\equiv 2R_0$   $\pm 2$  $\overset{\text{limit of }dt}{\text{equation}}\overset{\text{goint}_{\text{eff}}}{\text{d}t}$ <br> $\overset{\text{d}}{\text{d}t}$ <br> $\overset{\text{t}}{\text{t}}$  tion and  $\overset{\text{d}}{\text{d}t}$  as Taking the limit of *dt* going to zero, way have<br>how the number of  $\frac{1}{\sqrt{2N_C}}$  may heave<br>association and **di**ssociation and dissociation and the sociation of the sociation of the sociation of the sociation of the socia <sup>b</sup> <sup>C</sup>[ ]. (7) because only one molecule of dimer  $\frac{1}{\sqrt{2}}$ ,  $\frac{1}{\sqrt{2}}$ , and  $\frac{1}{\sqrt{2}}$ , change in the macroscope rate is matched to the manibe of points in an an upper bound given by a reaction will be detected in the matched by because only one molecule of dimer **Exceptible** Constrained. Summing 1<br>
Se Are  $\frac{1}{2}$  are unchanged and are un  $\frac{165}{160}$  and  $\frac{1}{160}$  and  $\frac{1}{160}$  and  $\frac{1}{160}$  and  $\frac{1}{160}$  and  $\frac{1}{160}$  are  $t + dt$  is the sum out, x, the appropriate Hill fu because only one molecule of dimer $\frac{1}{\sqrt{2}}$  of  $\frac{1}{\sqrt{2}}$  of the molecule box the number of pairs of a and B molecules is for  $\frac{1}{\sqrt{2}}$  of the central control of the central control of and sometimes will be propar only the dimer can bind ligand and signal downstream. The dimerization reaction is unusual. Let <sup>R</sup> denote <sup>a</sup> receptor and <sup>R</sup><sup>2</sup> denote <sup>a</sup> dimer of receptors. These species satisfy the reaction ceause only one molecule of dimer  $\frac{1}{\sqrt{2}}$   $\frac{1$ be only one molecule of dimer**ent and two molecules** systems in  $\mathcal{L}_{\text{g}}$  and  $\mathcal{L}_{\text{g}}$  are released by the number of pairs of A and B molecules in  $\mathcal{L}_{\text{g}}$  and  $\mathcal{L}_{\text{g}}$  are released by the multiplicatio  $\begin{minipage}{.4\linewidth} \textbf{on} & \textbf{non} \\ \textbf{on} & \textbf{non} \\ \textbf{on} & \textbf{non} \\ \textbf{on} & \textbf{non} \end{minipage}$ because the contract of the difference of  $\frac{1}{16}$  contract  $\frac{1}{16}$  contractions. Will be dissociation reactions will be dissociation reactions. The dimer,  $\frac{1}{16}$  constant  $\frac{1}{2}$  constant  $\frac{1}{2}$  constant  $\$  $\begin{array}{lll} \text{for} & \frac{\sqrt{2}}{\sqrt{2}}\text{ and } & \frac{\sqrt{2}}{\$ e over  $R_1^*$  une  $R_{21}^{t_6}$ . The number  $\Theta$  is the level of  $C$  molecules at a time  $t + dt$  is the time t plus the number gained in association reactions and minus the number gained in  $\sum_{i=1}^{k-1}$  and  $\sum_{i=1}^{k-1}$  and  $\sum_{i=1}^{k-1}$  as  $\sum_{i=1}^{k-1}$  and  $\sum_{i=1}^{k-1}$  and  $\sum_{i=1}^{k-1}$  and  $\sum_{i=1}^{k-1}$  and  $\sum$ interval of time dt, the association and dissociation reactions will b<br>solution in the set of the number of pairs of A and B molecules i<br>unplying that, refractory open  $h^{-2}$ unplying that<br>these pairs, will associate over  $R_1$  ime  $dt$ . The number of C molecules<br> $b d t N_C$ . Therefore, the number of C molecules at a time  $t + dt$  is the<br>time t plus the number gained in association reactions and minus  $\text{refractory}$  obey  $\text{open}$ how the number of  $\mathcal{C}_{\text{ABC}}^{\text{supp}}$  and  $\mathcal{C}_{\text{ABC}}^{\text{supp}}$  and  $N_C$ , chang over  $R_1^*$  time  $dt$ . The number of C molecule<br>mber of C molecules at a time  $t + dt$  is the  $\frac{d}{dx}$  and  $\frac{d}{dx}$  for  $\frac{d}{dx}$  and  $\frac{d}{dx}$  a  $\epsilon$  molec  $\sum_{n=0}^{\infty} \widetilde{R}^n \widetilde{R}^n$  (describe how the number  $\frac{d}{d}$   $\widetilde{R}^n \subset \mathcal{R}^{\underline{d}}$   $\widetilde{R}^{\underline{d}}$   $\widetilde{R}^n$  change because only one molecule of dimension-<br>  $\mathbf{X} = \mathbf{X} \mathbf{X} + \mathbf$ because only one molecule of dimer<br>
Many membrane receptors reversible dimerizations in the central of time dt, the association and dissociation reactions<br>  $\frac{\partial \mathbf{G}(\mathbf{K})}{\partial \mathbf{H}} = \frac{\partial \mathbf{G}(\mathbf{K})}{\partial \mathbf{H}} = \frac{\partial \mathbf{G}(\math$ because only one molecule of dimer **and signal downstream**<br>
is **Âure interior** to time dense to the number of pass of A and B molecule<br>
secretion of time dense to the anomalization reaction reaction with<br>  $\frac{1}{2}$   $\frac{1}{$ because only one molecule of dimer  $\frac{L}{L} \propto \frac{dR_0}{dR_0}$  and  $\frac{dR_0}{dR_0}$  ( $\frac{L}{dR_0}$  and  $\frac{dR_0}{dR_0}$  and  $\frac{dR_0}{dR_0}$  and  $\frac{dR_0}{dR_0}$  and  $\frac{dR_0}{dR_0}$  and  $\frac{dR_0}{dR_0}$  and  $\frac{dR_0}{dR_0}$  and  $\$  $\frac{1}{R}$ because only one molecule of dimer **and** of  $\mathbf{S} \subset \mathbf{z}$  are removed  $\mathbf{z}$  are removed  $\mathbf{z}$  are removed by  $\mathbf{z}$  are removed by  $\mathbf{z}$  and  $\mathbf{z}$  because the attention of the system and  $\mathbf{z}$  are remo because only one molecule of dimer $\blacksquare$  <br> as Articula dependent by the beam of the formula of the baread by the formula dependent by the baread by the baread by the association and the association. Although the associati because only one molecule of dimer  $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$  of time dt, the association and dissociation reactions will be considered at the r mber of  $C$  m<br>ined in assoc<br> $\sum_{k \geq 0} \frac{k-2}{k}$ <br> $\sum_{k \geq 0} \frac{k}{k}$  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2$ because only one molecule of direct **unicely**<br>similarly control in the second into and distribution, we can consider the<br>similar involved in the molecules are interested in the dimersion of distributions. The<br>interesting e over  $R_1^*$  +  $R_2^*$  =  $\frac{1}{2}$  constant  $=$   $R_0^*$  +  $2R_2^*$  of  $\frac{1}{2}$  =  $\frac{1}{2}$  constant  $t + dt$  is the Summing 1<br>  $N_C$ , change<br>
tions will b<br>
molecules i<br>  $C$  molecules<br>  $\begin{array}{l}\nC \text{ molecules} \\
\text{left} \\
\text{left} \\
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\text{left}\right \\
\text{limits the}\n\end{array}$ <br>  $\begin{array}{l}\n\text{mmax} \\
\text{time} \\
\text{$ because only one molecule of dimer **forms** of dimer **only of**  $\frac{1}{2}$  and  $\frac{1}{2}$  also summing  $\frac{1}{2}$  and  $\frac{1}{2}$  because only one m<br>  $\frac{X}{\text{S}}$  is the concentration of A and  $\frac{X}{\text{S}}$ <br>  $\frac{X}{\text{S}}$  is the contract of A and  $\frac{X}{\text{S}}$ <br>  $\frac{X}{\text{S}}$  is the num<br>  $\frac{X}{\text{S}}$  is the num<br>  $\frac{X}{\text{S}}$  is the concentration of B.<br> to<br>cause only one nucleate of direct **Lab**ables. So SS AVE different of the<br>section and the macroided C rate of the macroscopic rate of the macroscopic<br>difference is different to the macroscopic rate b are unchefored and ber of pairs of  $eA_n$  and  $B$  molecules<br>  $\prod_{i=1}^{n} P_{K_i}^{H_i}$ . The number of  $C$  molecules<br>  $C$  molecules at a time  $t + dt$  is the<br>  $C$  molecules at a time  $t + dt$  is the<br>  $\lim_{m \to \infty} e^{-x}$ <br>  $\frac{y(x)}{y_{\text{max}}} = \frac{x^n}{K^n + x^n}$ <br>
or o because only one molecule of dimer<br>  $\frac{1}{2}$ ,  $\frac{1}{2}$ because only one molecule of time **can**  $\partial G$  given  $\partial G$  and  $\partial G$  given by  $\partial G$  and  $\partial G$  and  $\partial G$  and  $\partial G$  molecule is unitary  $\partial G$  and  $\partial G$  and  $\$ because only one molecule of dimer **ED** or  $\hat{\mathcal{R}}$  denoted by consistent and R consistent and R and R molecules is a result of  $\hat{\mathcal{R}}$  denoted by the reservoir on and species satisfy the reaction of reaction  $\hat{\mathcal{$ because only one molecule of dimer  $\frac{\sqrt{2}}{\sqrt{2}}$   $\frac{\sqrt{$ because only one molecule of dimer **and set of the first of**  $\mathcal{S}_1$  reaction incredible for  $\mathcal{S}_2$  reaction. Although the property of the property of  $\mathcal{S}_2$  reaction. Although the association of  $\mathcal{S}_2$  reactio  $\begin{array}{l} N_C, \text{ change} \ \text{ons will be} \ \text{models} \ \text{in} \ \mathcal{F} \ \text{implg} \ \text{in} \ \mathcal{F} \ \text{in} \ \mathcal{F} \ \text{in} \$ ] and the dissociation reaction proceeds at the rate b R[ se only one molecule of dimer  $\overline{L}$  is  $\overline{G}$  multiplates. Summing and  $\overline{G}$  is  $\overline{G}$   $\overline{G}$   $\overline{G}$   $\overline{G}$   $\overline{G}$   $\overline{G}$   $\overline{G}$   $\overline{G}$  and  $\overline{G}$  for time *dt*, the association and *dissociatio* because only one molecule of dimer  $\frac{d}{dx}$  is  $\frac{d}{dx}$  in the  $\lambda$  is  $\lambda$  summing l<br>  $\mathbf{B}$   $\mathbf{B}$   $\mathbf{B}$   $\mathbf{B}$   $\mathbf{B}$   $\mathbf{C}$  and  $\mathbf{C}$  and  $\mathbf{C}$  and  $\mathbf{C}$  are discociation and  $\mathbf{C}$   $\mathbf{B}$  time t plus the number gained in association reactions and minus the i<br>reactions dimer because only one molecule of dimer  $\frac{\overbrace{\text{of}}^2 \text{bs}}{\text{refactors}}$   $\frac{\overbrace{\text{of}}^2 \text{bs}}{\text{refactors}}$  Summing 1 these pairs will associate over a time  $H^2$  and  $\overline{H}^2$  and  $\overline{H}$  $T_{C}(\mathbf{k}_{2} + u_{l}) = \frac{\text{refract}}{\text{refactor}}$ <br>Tolding the limit of *H* going to going we have and statisfy Eq. 33, the system is using the system is using the correction of the cycle to  $\sim$  $\Gamma$ refractory states. In the refractory state  $\frac{1}{4} \frac{1}{2} \frac{1}{N} \frac{1}{2} \frac{1}{N} \frac$ association and  $d\text{t}$  is  $\frac{1}{4}$   $\frac{1}{2}$   $\frac{1}{2}$  association and  $d\text{t}$   $\text{isocia}$   $\text{t}$   $\text{t}$   $\$  $1.1$  Chemical rate equations  $\mathcal{L}^{\text{max}}_{\text{max}}$ because only one molecule of dimer of  $\frac{1}{\sqrt{2}}$  is  $\frac{1}{\sqrt{2}}$ . Summing 1<br>  $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$ time t plus the number gained in association reactions and minus the  $\frac{1}{2}$  $M$  membrane receptors receptors receptors receptors receptors receptor-receptor-receptor-receptor-receptors receptors  $\mathcal{L}$  $\text{truncor} \text{dim} \text{tr} \left[ \text{of} \text{Im} \text{R}_{\text{C}} \text{dim} \right] + \text{Ind} \text{Im} \text{R}_{\text{C}} \text{dim} \text{R}_{\text{C}} \text{dim} \text{R}_{\text{C}}$  $\sum_{\text{refraction}} W_{\text{c}}(k_{2}^{\text{min}}dt) = \sum_{\text{open}} W_{\text{c}}(k_{2}^{\text{min}}dt)$ Taking the limit of dt going to zero, we have becance and the concentration of A and E and I ummaterial concentration of B. III is the conc because only one melocule of dimensional contract  $\mathcal{L}$ because only one molecule of dimer **of the set of**<br>  $\mathbf{3S} \mathbf{\mathcal{H}}$   $\mathbf{R} \mathbf{B}$   $\mathbf{B} \mathbf{B}$   $\mathbf{B} \mathbf{B}$  and  $\mathbf{B} \mathbf{B}$  are numbered interval of time dt, the association and  $\mathbf{d}\mathbf{b}$ <br>  $\mathbf{S} \mathbf{Q} \mathbf{$ because only one molecule of dimer<br>  $\frac{1}{2}$  Sarte and interval of three div, the mass origins how the manuform<br>  $\frac{1}{2}$  interval of the matrix of the manuform of the matrix of the matrix of the matrix of the matrix of because only one molecule of dimer $\blacksquare$  is explicitly dimerial and sometime relationships the set of the sometimes of minimal angle in the sometime relation of the sometime relation of the sometime relation of the someti because only one moleculo of since  $\frac{1}{2}$ ,  $\frac{1}{2}$ , because only one molecule of dimer **Lab** special species sets and  $\frac{1}{2}$  of  $\frac{1}{2}$  contains and  $\frac{1}{2}$  contains a receptor and  $\frac{1}{2}$  contains a receptor  $\frac{1}{2}$  contains a receptor  $\frac{1}{2}$  contains a reac because only are another of the rate of  $\epsilon$  and  $\epsilon$  are removed by the removed of  $\epsilon$  are removed by  $\epsilon$  are removed by  $\epsilon$  and  $\epsilon$  are removed by  $\epsilon$  and  $\epsilon$  are removed by  $\epsilon$  and  $\epsilon$  are removed by  $\epsilon$  and because only as considered although **Excelsion** and the specific  $S$  are released by  $\frac{1}{2}$  and  $\frac{1}{2}$  cause only one molecule of dimer **interestigation** S. Summing 1<br> **And the distribution reaction and distribution** reactions will be<br>
distributed at the association and dissociation reactions will be<br>
and  $B$  molecules int and associate identity of pairs of A and B molecules is<br>  $d_2^k = \frac{1}{\text{Ronsumper}} \frac{\text{er}}{\text{Re}} \left( \frac{C}{C} \frac{PQ}{PQ} \right) \text{Re} \text{u} \text{u}}{\text{E}^k}$ <br>
rolecules at a time  $l + dt$  is the<br>
ciation reactions and minus the :<br>
ciation reactio Many membrane receptors of the many membrane receptors in the experimental interest of the sometimes relation and sometimes relationships of the sometimes relation of the sometimes relation of the sometimes relationships because of the signal properties in the canonical downstream.<br>
So figure for the signal downstream can be dimerization reaction reaction reaction reaction reaction is unusual.<br>  $\frac{\partial f(x)}{\partial x} = \frac{\partial f(x)}{\partial x} + \frac{\partial f(x)}{\partial y}$  and  $\$ because the out particle of receptor  $\frac{1}{2}$  denote a receptor  $\frac{1}{2}$  denote a receptor  $\frac{1}{2}$  denote a reaction on the reaction of reaction and Receptor and Receptor and Receptor and Receptor and Receptor and Rec tensor only one understand does **Electric Constitute** of R are removed and the rate of  $\frac{1}{2}$  and  $\frac{1}{2}$  are removed and  $\frac{1}{2}$  are removed and  $\frac{1}{2}$  are removed and  $\frac{1}{2}$  are removed and  $\frac{1}{2}$  are re be one only are relay<br>the of the modellic of distribution and the set of the first set of the<br>set of reaction and the first are relation and the properties are released<br>in the first point of a reaction and the molecules i because only one molecule of dimer<br>  $\sum_{i=1}^{\infty} \frac{1}{2}$  and  $\sum_{i=1}^{\infty} C_i$  and  $\sum_{i=1}^{\infty$ e over  $R$  time  $dt$ . The number of C molecule<br>umber of C molecules at a time  $t + dt$  is the  $\frac{d}{dR}$   $\frac{d}{dR}$   $\frac{d}{dR}$  and  $\frac{d}{dR}$  are released at a time  $\psi$  +  $\frac{d}{dR}$  is the time  $t$  plus the number gained in association reactions and minus the intervals of dimensional  $\frac{d}{dR}$   $\frac{d}{dR}$   $\frac{d}{d$ because only one molecule of din<br>  $\mathcal{S}\mathbf{S}$   $\mathbf{A}$   $\mathbf{E}\mathbf{B}$   $\mathbf{B}\mathbf{B}$   $\mathbf{B}\mathbf{B}$  and  $\mathbf{B}\mathbf{B}$  are different on the interval of time  $dt$ , the associations of  $\mathbf{B}\mathbf{B}\mathbf{B}$   $\mathbf{B}\mathbf{B}$   $\mathbf{B}\math$ s only one molecule of dimer **Example 19**  $\mathbb{Q}$  summing l<br> **R (A)**<br> **b** in the *s* are incrediction and dissolution reactions with b<br> **A)**<br> **A)**<br> **C** in the section and also set in the section in the section of pairs  $\epsilon_{\text{R}}$   $\epsilon_{\text{R}}$   $\epsilon_{\text{R}}$  and  $\epsilon_{\text{R}}$  is the number of pairs of A and B molecules is implying that<br>these pays will associate over  $R^i$  ting  $dt^i$ . The number of  $C$  molecules<br> $\epsilon_{\text{R}}$   $\epsilon_{\text{R}}$  =  $\epsilon_{\text{R}}$  because only one molecule of dimer **Election** of dimer **Election** S. Summing <br>
S. **Area (Figure of the monod of dissociates** and the second intervals in the second of twice Eq. 9 gives  $\frac{1}{2}$  and  $B$  molecules is an  $\$  $\vec{b}$  deN<sub>C</sub>. Therefore, the number of C molecules at a time  $t^{0} + dt^{0}$  is the<br>time t plus the number gained in association reactions and minus the is  $\sum_{i=1}^{\infty}$  and  $\sum_{i=1}^{\infty}$  and  $\sum_{i=1}^{\infty}$  and  $\sum_{i=1}^{\infty}$  and  $\sum_{i=1}^{\infty}$  and  $B$  molecules is  $\sum_{i=1}^{\infty}$  and  $\sum_{i=1}^{\infty}$  and  $\sum_{i=1}^{\infty}$  molecules is the space over  $\sum_{i=1}^{\infty}$  time  $\sum_{i=1}$  $\frac{1}{\sqrt{N}} \frac{1}{\sqrt{N}} \frac{1}{\sqrt{N}}$  $\max_{\mathbf{p}}\left(\overrightarrow{\underbrace{\mathbf{q}}_{\mathbf{k}_2}}\mathbf{H}_{\mathcal{U}}\right)$ because only one moles because only one molecule of dimer  $\frac{1}{4}$  is differentially  $\frac{1}{2}$  interaction and dissociation reaction and  $\frac{1}{4}$  interaction and  $\frac{1}{4}$  in the second and  $\frac{1}{4}$  interaction  $\frac{1}{4}$  in the macroscopic r Meridian considerate of the thermal model of  $\mathbf{R} = \mathbf{R}$  and  $\mathbf{R} = \mathbf{R}$  because only one molecule for time  $\frac{1}{2}$  or  $\frac{1}{2}$  or  $\frac{1}{2}$  only  $\frac{1}{2}$  or  $\frac{1}{2}$  of  $\frac{1}{2}$  on  $\frac{1}{2}$  or  $\frac{1}{2}$  on  $\frac{1}{2}$  or  $\frac{1}{2}$  or  $\frac{1}{2}$  or  $\frac{1}{2}$  or  $\frac{1}{2}$  or  $\frac{1}{2}$  or  $\$ become only one molecule of direct **LED**  $\frac{1}{2}$  denoted  $\frac{1}{2}$  conducts in  $\frac{1}{2}$  denoted  $\frac{1}{2}$  conducts  $\frac{1}{2}$  denoted  $\frac{1}{2}$  conducts  $\frac{1}{2}$  and  $\frac{1}{2}$  conducts  $\frac{1}{2}$  and  $\frac{1}{2}$  conducts The dt, the association and **dissociation** re<br> **Rightary** discussion and **dissociation** re<br> **Rightary** dissociate over  $R_1^*$  image.<br> **Rightary** dissociate over  $R_2^*$  in the number of  $R_3^*$  in the constant in<br>
herefo From a strip considered into the rate of  $\mathbb{R}^n$  and  $\mathbb{R}^n$  are response to the removed of  $\mathbb{R}^n$ , the results of  $\mathbb{R}^n$  and  $\mathbb{R}^n$ , the rate of  $\mathbb{R}^n$  and  $\mathbb{R}^n$  are results of  $\mathbb{R}^n$ . The by the first particle is the form **in** order to provide the control of the state of the first particle is the first particle in the first particle is the first particle in the first particle in the breaction and the state polecule of dimer **and solution**  $\mathbf{R}^T \mathbf{Q}$  we discontribute the association and  $\mathbf{d}^T \mathbf{S}$  consistents at the sesociation reactions will be consistent that  $\mathbf{R}^T \mathbf{S}$  and  $\mathbf{Z}$  means of  $\mathbf{A}$  and while mumber of pairs of  $\epsilon A_0$  and B molecules<br>e over  $R_1^*$  time  $R_2^*$ . The number of C molecule<br>umber of C molecules at a time  $t + dt$  is the nming ]<br>
change<br>
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will l because only one collecules at dimer **and**  $\frac{1}{\sqrt{2}}$  in the dimersion of the dimersion of the collections.<br> **SAPRAMES** are involved in the stations of the dimersion of the dimersion of the dimersion of the dimersion of be  $\frac{1}{35}$   $\frac{1}{35}$   $\frac{1}{35}$   $\frac{1}{65}$   $\frac{1}{65}$ the same only the matched of these **limited.**  $\frac{1}{2}$  which is distinguished. The unit of the matched and are unchanged and are s Measure and y the membrane of dimer **united of** dimering  $\frac{1}{2}$ ,  $\frac{1}{2}$ , beams any one redestric down **ED**-orginal downstreams of the dimerization of dimerization reaction and distance reaction is unusual.<br> **Solution** is unusual. The analog of the signal downstream. The dimerization reaction i Let us consider the controller of the **LEt A** denote a receptor  $\frac{1}{2}$  denote a receptor  $\frac{1}{2}$  and  $\frac$ because only one and<br>entity of three contents of dimersions for  $\frac{1}{2}$  ( $\frac{1}{2}$ <br>
in the rate in the rate interest in the number of  $\frac{1}{2}$  ( $\frac{1}{2}$ <br>  $\frac{1}{2}$ ),  $\frac{1}{2}$  ( $\frac{1}{2}$ ),  $\frac{1}{2}$  ( $\frac{1}{2}$ ),  $\frac{1$ because only one molecule of dimes **lines**  $\frac{1}{\sqrt{2}}$  of  $\frac{1}{2}$  contains are released by the formula are released by the b reaction correlation and the association. Although the b reaction correlation and the associa because only one molecule of dimer  $\frac{f_{\text{max}}}{\sqrt{2}}$  discovered at the rate b  $\frac{f_{\text{max}}}{\sqrt{2}}$  and  $\frac{f_{\text{max}}}{\sqrt{2}}$  and  $\frac{f_{\text{max}}}{\sqrt{2}}$  and  $\frac{f_{\text{max}}}{\sqrt{2}}$  and  $\frac{f_{\text{max}}}{\sqrt{2}}$  and  $\frac{f_{\text{max}}}{\sqrt{2}}$  and  $\frac{$ be of dimer  $\frac{1}{\log(\frac{1}{\log n})}$  of  $\frac{1}{\log(\frac{1}{\log n})}$  of  $\frac{1}{\log(\frac{1}{\log n})}$  of  $\frac{1}{\log(\frac{1}{\log n})}$  or  $\frac{1}{\log(\frac{1}{\log n})}$  or  $\frac{1}{\log(\frac{1}{\log n})}$  and *B* molecules in the number of  $C$  molecules  $\frac{1}{\log(\frac{1}{\log n})}$  and e molecule of dimer  $\frac{1}{\sqrt{2}}$  because  $\frac{1}{\sqrt{2}}$   $\frac{1}{\sqrt{2}}$ where stress and  $\alpha$  and  $\alpha$  respective to the exploration.  $D$  model can recept  $c$  and  $c$  $\frac{d}{dt} \frac{d}{dt} \frac{$ Eq. imperiment in the number of  $A$  and  $[R]$   $\downarrow R$  for  $[R_2]$  = constant = er of  $C$  molecules at a time are removed R The removed R The rate equations for this system are atypical because two molecules of the rate  $\sim$ refractive to the associate over  $[R]$  with  $[R_2]$  = constant  $\cong$   $[R]_0$  +  $\cong$   $[R_2]_0$ <br>bde $N_C$ . Therefore, the number of C molecules at a time  $t + dt$  is the<br>time t plus the number gained in association reactions and mi katory<br>15  $\sum_{n=1}^{\infty}$   $\mathbb{R}^n$   $\mathbb{R}^n$   $\mathbb{R}^n$   $\mathbb{R}^n$  and  $\mathbb{R}^n$  and  $\mathbb{R}^n$   $T$  is said to undergo a the independent of  $C(\frac{1}{k_2} + \frac{1}{k_1})$  and  $C(\frac{1}{k_2} + \frac{1}{k_2})$  and  $N_A N_B$  and  $N_A N_B$  $\omega$ ssoutiant mussouti $\omega$  is a constraint on the reaction rates:  $\text{Row}$  channels  $\overline{S_4} \not\cong \frac{\text{div}}{\text{div}} \overline{S_0}^{\text{1}} \overline{C_2}$ , enough  $\alpha$  refractive  $\alpha$  in the refraction rarely  $\alpha$  is the channel rate. Into  $\alpha$  is a reference into  $\alpha$  in  $\alpha$  is a reference into  $\alpha$  in  $\alpha$  is e over  $R_1^{\text{th}} + 2[R_2^{\text{th}}] = \text{constant} \equiv 2[R_0^{\text{th}} + 2[R_2]_0^{\text{th}}]$ <br>umber of C molecules at a time  $t + dt$  is the and  $\alpha$  complex  $\alpha$  reception  $\mathbb{R}^N$  and  $\alpha$  is  $\alpha$  $\begin{bmatrix} \text{The num} \\ \text{Comstant} \end{bmatrix}$  $\sum_{\text{reflex}}$   $\sum_{\text{coeff}}^{\text{reflex}}$   $\sum_{\text{coeff}}^{\text{reflex}}$   $\sum_{\text{coeff}}^{\text{reflex}}$   $\sum_{\text{coeff}}^{\text{reflex}}$   $\sum_{\text{eff}}^{\text{reflex}}$  and  $\sum_{\text{eff}}^{\text{reflex}}$   $\sum_{\text{eff}}^{\text{reflex}}$  and  $\sum_{\text{eff}}^{\text{reflex}}$  and  $\sum_{\text{eff}}^{\text{reflex}}$  and  $\sum_{\text{eff}}^{\text{reflex}}$  and  $\sum_{\text{eff}}$ association and dissociation redetions will b<br>which mumber of pairs of Anand B molecules<br>e over  $R_1$  time  $dt$ . The number of C molecule<br>umber of C molecules at a time  $f + dt$  is the because only one mole<br>  ${}^{18}S^5$   ${}^{6}R^6$  is the concentration of time dt, the<br>  ${}^{6}R^8$  ${}^{6$ because only one molecule of dimer $\overline{[\textbf{off}]}$ ns $\overbrace{\textbf{off}^{\text{in}}_{\textbf{off}}}$  disk, S because only one molecule of dimer **lot** instantial factors. Summing<br>
25  $\hat{A} \times B$  in  $\hat{B} \times C$  less tries how the number of  $\hat{B} \times C$   $\hat{B}$   $\hat{C}$   $\hat{B}$   $\hat{C}$   $\hat{C}$   $\hat{C}$   $\hat{C}$   $\hat{C}$   $\hat{C}$   $\hat{C}$   $\hat$ therause ody one molecule of those **and are unchanged and are unchanged** and are unchanged and are unchanged and are unchanged and are uncertainty of the macroscopic rate b are uncertainty of the matrix of the matrix of t Many members only are molecule of dones. **However only a** significantly section in the control of the sometimes of an exception of an exception of the control of because only one moderns of since and interesting  $\theta$ . And  $\theta$  and Let  $R$  denote a receptor and R2 denotes shown and  $R$  is the reaction of  $R$  is a reaction of  $R$  and  $R$  an based in the seasontan and dissociation reaction reaction in the seasontan and dissociation reaction reaction reaction reaction reaction reaction reaction reaction in the rate of Division reaction reaction is the rate of Nexus are considered chaos  $\frac{\sum_{i=1}^{n} \hat{S}_{i}^{(i)} \sum_{j=1}^{n} \hat{S}_{j}^{(i)} \sum$ because solve as molecules at since  $\frac{1}{2}$  reaction and the formula in the b reaction.<br> **Solution**  $\frac{1}{2}$  reaction and the since  $\frac{1}{2}$  reaction. Although the association and the b reaction. Although the associat because only one molecule of dimer $\frac{\sum_{i=1}^{\infty} \frac{1}{2} \sum_{i=1}^{\infty} \$ <sup>18</sup><br>R<br>Ile because only one molecule of dimer of the  $\mathcal{L}_{\text{initial}}$  and  $\mathcal{L}_{\text{total}}$ <br>  $\mathcal{L}_{\text{total}}$   $\mathcal{L}_{\text{total}}$   $\mathcal{L}_{\text{total}}$ <br>  $\mathcal{L}_{\text{total}}$   $\mathcal{L}_{\text{total}}$   $\mathcal{L}_{\text{total}}$  (Alescribe how the number  $\mathcal{L}_{\text{total}}$   $\mathcal{L}_{\text{total}}$  is inte be of dimer **Lab** of  $\overline{\phi}$  is a symphonic symphon of a section and dissolved<br>for monder of pairs of A and D molecules it<br>the multier of pairs of A and D molecules it<br>metals are involved in a model in the dimer,  $\overline{\phi}$ ] (9) 2 e molecule of dimer **labered** of  $\frac{1}{2}$  one molecule of  $\frac{1}{2}$  one molecule and  $\frac{1}{2}$  one molecule and  $\frac{1}{2}$  molecule and  $\frac{1}{2}$  molecules. Such an  $\frac{1}{2}$  molecule or policies of A and  $B$  molecules is umber of  $\mathcal{C}$  molecules at a time  $t + dt$  is the which is an extendent of a distribution value  $\frac{1}{2}$  and  $\frac{1}{2}$  more curres<br>cover  $\frac{1}{2}$  the number of  $\frac{1}{2}$  more will  $\frac{G_1}{\mathbf{Q}}\sum_{i=1}^{K} \frac{G_2}{\mathbf{Q}}\left(\sum_{i=1}^{K} \frac{G_1}{\mathbf{Q}}\right)$  and  $\frac{G_2}{\mathbf{Q}}\left(\sum_{i=1}^{K} \frac{G_2}{\mathbf{Q}}\right)$  and  $\frac{G_1}{\mathbf{Q}}\left(\sum_{i=1}^{K} \frac{G_1}{\mathbf{Q}}\right)$  and  $\frac{G_2}{\mathbf{Q}}\left(\sum_{i=1}^{K} \frac{G_1}{\mathbf{Q}}\right)$  and  $\frac{G_$  $\text{UIC}^{\bullet} \text{OT}^{\bullet}$  dimer  $\text{Corr}_{\text{refractory}}^{\bullet}$ <br>Taking the limit of dt going-t stochalated interval in the number of pairs of A and B molecules in<br>unplying that<br>**d**, DIOXIMILL associate over  $R \neq R_2$  = constant =  $R|_{0} +$ ,  $R|_{0}$  =  $R$  $\frac{1}{2}$   $\frac{1}{2}$   $\frac{1}{2}$   $\frac{1}{2}$   $\frac{1}{2}$   $\frac{1}{2}$   $\frac{1}{2}$  $\frac{1}{2}$  because only one molecule of  $\frac{1}{2}$  $\frac{1}{2}$  by the number of  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  denoting that  $\frac{1}{2}$  ( $\frac{1}{2}$ ).  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2$ time t plus the number gained in association reactions and minus the interestions.<br>
The of dimerment of  $\frac{k+2}{(k+1)!}$  is  $\frac{1}{(k+1)!}$  in  $\frac{1}{(k+1)!}$  in  $\frac{1}{(k+1)!}$  in disconnections in disconnections. which a number of pairs of  $A<sub>q</sub>$  and  $B$  molecules  $\alpha$  is the cause only one molecule of dimer  $\delta$ . because only one molecule of dimer  $\frac{1}{16\pi}$  steerable  $\alpha$ . Summing is  $\hat{S}$   $\hat{M}$  is  $\hat{M}$  (the *dt*, the association and dissociation reactions will be such that  $\alpha$  the macroscope in  $\alpha$  and  $B$  molecules because only one molecule of dimer  $\frac{1}{\sqrt{2}}$  as  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$ . Summer  $\frac{1}{2}$  and  $\frac{1}{2}$  of time  $dt$ , the association and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $B$  moleculus  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1$ becau<br>
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what because only one molecule of dimer $\frac{\sqrt{2}}{\sqrt{2}}$  and  $\frac{\sqrt{2}}{\sqrt{2}}$  a because only one molecule of dimer $\frac{1}{k}$  so  $\frac{1}{k}$  so  $\frac{1}{k}$  so  $\frac{1}{k}$  so  $\frac{1}{k}$  so  $\frac{1}{k}$  and  $\frac{1}{k}$  because only one molecule of dimer  $\frac{1}{\sqrt{2}}$  of  $\frac{1}{\sqrt{2}}$  of  $C$ , change function of time dt, the association and  $\frac{1}{\sqrt{2}}$  of  $C$ , change function  $C$  is the reaction of  $A$  and  $B$  molecules is  $\frac{1}{\sqrt{2}}$  or The method of  $R_f$  of  $R_e$  is  $\alpha$ <br>e number  $R_f$   $\alpha$   $R_e$   $\alpha$ <br>e number  $R_f$   $\alpha$   $R_e$   $\alpha$ <br>e number  $R_f$   $\alpha$   $\alpha$   $R_f$   $\alpha$ because only one molecule of dimer  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$  of  $\frac{1}{\sqrt{2}}$  or the *R*, the association and dissociation receives will be attended to  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$  or  $\frac$ because only one molecule of dimer **and set of** the municipality of the molecules in the formula of the section and differential reactions will be reacted by the section of anisotechic property. The annotation is property row the number<br>association and **dissociate**<br>which a number of pairs of a<br>e over  $R_1^3 + 2[R_2^2] = \text{const.}$ <br>umber of C molecules at<br>increases with increasing levels<br> $\frac{y(x)}{y_{\text{max}}} = \frac{y}{K^n}$  $\frac{d}{d\mathbf{B}}$  and  $\frac{d}{d\mathbf{B}}$  and  $\frac{d}{d\mathbf{B}}$  and  $\frac{d}{d\mathbf{B}}$  and  $\frac{d}{d\mathbf{B}}$  and  $\frac{d}{d\mathbf{B}}$  and  $\frac{d}{d\mathbf{B}}$  measured density reactions will be of pairs of A and B molecules is  $\frac{d}{d\mathbf{B}}$ . The numb  $\begin{array}{l} \mathop{\mathrm{ang}} \ 1 \ \mathop{\mathrm{blue}} \ \mathop{\mathrm{all}} \ \mathop{\mathrm{blue}} \ \mathop{\mathrm{blue}} \ \mathop{\mathrm{blue}} \ \mathop{\mathrm{blue}} \ \mathop{\mathrm{blue}} \ \end{array}$ because the molecule of dimer  $\frac{1}{\sqrt{2}}$ ,  $\frac{1}{2}$ ,  $\begin{bmatrix} 1 & \frac{1}{2} & \frac{1}{2} & \frac{1}{2} \\ 0 & \frac{1}{2} & \frac{1}{2} \\ \frac{1}{2} & \frac{1}{2} & \frac{1}{2} \end{bmatrix}$  =  $\begin{bmatrix} 1 & \frac{1}{2} & \frac{1}{2} & \frac{1}{2} \\ 0 & \frac{1}{2} & \frac{1}{2} & \frac{1}{2} \\ 0 & \frac{1}{2} & \frac{1}{2} & \frac{1}{2} \\ 0 & \frac{1}{2} & \frac{1}{2} & \frac{1}{2} \end{bmatrix}$  =  $\begin{bmatrix} 1 & \$ ente of dimer **leasting one of diffuse only one models** of  $\zeta$  redifferences on the number of pairs of  $A$  and  $B$  molecules is  $\eta$ . The number of pairs of  $A$  and  $B$  molecules is  $\eta$  in  $\eta$  in  $\eta$  if  $B$ ,  $\eta$  in Convert Eq. 3 into an extended the EQ<sub>50</sub> of the number of C, we must divide the concentration of C, we must describe of concentration of C, when the control of C, we must divide of the concentration of C, we must divide **EXAMPLE INTERFORMALE IN AN INTERFORMALE IN THE CONCENTRATION**  $\frac{d}{dt}$  is the concentration of A and  $\frac{d}{dt}$  is the concentration of B. If we define the concentration of  $\frac{d}{dt}$  is the concentration of B. If we defi  $\tilde{0}$ lect նne<br>:⊶։ because c<br>  $\frac{1}{2}$ S  $\tilde{A}^{\mu}$ <br>  $\frac{1}{2}$  interval c<br>  $\frac{1}{2}$  s  $\tilde{C}^{\mu}$  and  $\tilde{C}^{\mu}$ <br>  $\frac{1}{2}$   $\tilde{C}^{\mu}$   $\tilde{C}^{\mu}$ <br>  $\tilde{C}^{\mu}$   $\tilde{C}^{\mu}$ <br>  $\tilde{C}^{\mu}$  time t plus<br>  $\tilde{C}^{\mu}$  Taking the because only one molecule of dimer $\blacksquare$  of the molecule paper is a set of the molecule in the set of the molecule in the set of the molecule because only one molecule of time<br>  $\frac{1}{1000}$  is diffused. The units of  $\frac{1}{1000}$  is diffused and<br>
independent into the manufacture of the macroscopic change<br>  $\frac{1}{1000}$  is diffused and are unchanged and are unchan because only one molecule of dimer<br>
S. APRAMAGE certic how the number of  $\mathcal{G}_1$  conditions relations will be<br>
force of order than the contain and discolation read in not contain the<br>
form a receptor-receptor of the num because only one molecule of dimer  $\overline{\text{L}}$  socialization reactions will be dimerized and the secondition and discontinue needed in continue with  $\frac{\text{m}}{\text{m}}$  and  $\overline{\text{m}}$  and  $\overline{\text{m}}$  and  $\overline{\text{m}}$  and  $\overline{\text{m}}$ because only one molecule of times  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  or  $\frac{1}{\sqrt{2}}$  and  $\frac{1}{\sqrt{2}}$  and because only one molecule of dimer  $\frac{1}{\sqrt{6}}$   $\frac{$ by the molecule of dimer<br>  $\sum_{k=1}^{\infty} \frac{1}{k!} \sum_{k=1}^{\infty} \frac$  $\mathbf{R}$  and the disconnection  $\frac{\mathbf{G}^{\mathbf{F}}_{\mathbf{R}}\mathbf{B}^{\mathbf{F}}_{\mathbf{R}}\mathbf{H}}{\mathbf{R}^{\mathbf{F}}_{\mathbf{R}}\mathbf{B}^{\mathbf{F}}_{\mathbf{R}}\mathbf{B}^{\mathbf{F}}_{\mathbf{R}}\mathbf{C}}$ . Summing let the rate be rate by the number of  $\mathbf{R}^{\mathbf{F}}_{\mathbf{R}}$   $C$  $\begin{array}{l} \frac{1}{2} \left( \frac{1}{2} \frac{1}{2}$ because only one model of  $\frac{1}{k}$  on  $\frac{1}{k}$  on  $\frac{1}{k}$  and  $\frac{1}{k}$  on  $\frac{1}{k}$  and  $\frac{1}{k}$  and  $\frac{1}{k}$  and  $\frac{1}{k}$  and  $\frac{1}{k}$  on  $\frac{1}{k}$  or  $\frac{1}{k}$  or  $\frac{1}{k}$  or  $\frac{1}{k}$  or  $\frac{1}{k}$  or  $\frac{1}{k}$  $\alpha \propto [R] + 2[R_2] = \text{constant} \approx [R]_0 + 2[R_2]$ reactions: dimer  $\frac{G}{\sqrt{K}}\left[\frac{1}{K}\right]$  $\frac{1}{2}$  and  $\frac{1}{2}$  receptors. The reaction  $\frac{1}{2}$  receptor  $\frac{1}{2}$  and  $\frac{1}{2}$  receptor  $\frac{1}{2}$  receptor  $\frac{1}{2}$  receptor and  $\frac{1}{2}$  receptor  $\frac{1}{2}$  receptor  $\frac{1}{2}$  receptors.  $\mathcal{L}_2^{\text{ref}}$   $\mathcal{L}_2^{\text{ref}}$   $\mathcal{L}_3^{\text{ref}}$   $\mathcal{L}_4^{\text{ref}}$   $\mathcal{L}_5^{\text{ref}}$   $\mathcal{L}_6^{\text{ref}}$   $\mathcal{L}_7^{\text{ref}}$   $\mathcal{L}_8^{\text{ref}}$   $\mathcal{L}_7^{\text{ref}}$   $\mathcal{L}_8^{\text{ref}}$   $\mathcal{L}_7^{\text{ref}}$   $\mathcal{L}_8^{\text{ref}}$   $\mathcal{L}_9^{\text{ref}}$   $\mathcal{L}_7^{\text{ref$ because only one molecule of dimer  $\frac{\mathbf{f} \cdot \mathbf{f}}{k_2}$   $\mathbf{f} \cdot \mathbf{f}$   $\mathbf{f}$  and  $\mathbf{f} \cdot \mathbf{f}$   $\mathbf{f}$  and  $\mathbf{f} \cdot \mathbf{f}$   $\mathbf{f}$  and  $\mathbf{f} \cdot \mathbf{f}$  and  $\mathbf{f} \cdot \mathbf{f}$  and  $\mathbf{f} \cdot \mathbf{f}$  $\text{proxmin}$  through  $\text{arg}(R_1^* + 2|R_2^*| = \text{constant} = |R|_0^{\circ} + 2|R_2^*|_0^{\circ}$ If it is the function of the model each of the  $t + ut$  is the  $\frac{1}{2}$  the limit of  $dt_1$  god  $g$  and  $g$  are  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  arbitrarily be a rate constants cannot be a rate constants can not be a rate constants can not be a rate constants of  $\frac{1}{2}$  and  $\$ how the number of  $\tilde{C}$  going regules  $_0N_C$ , change association and  $\overline{df} + 2\overline{dg} = 0$  or write  $\overline{dg}$  will k state allows the particular order through the plane, and the pass through the rate for the rate field of the rate how the number of  $\mathcal{C}'$ ,  $\mathcal{W}$   $\mathcal{R}$  is  $N_C$ , chang  $\widetilde{\mathfrak{gl}}$   $\widetilde{\mathfrak{gl}}$   $\widetilde{\mathfrak{gl}}$   $\widetilde{\mathfrak{gl}}$   $\widetilde{\mathfrak{gl}}$  associate over  $R$  the  $R$  membrane and the cell molecule and the cell member these pairs will associate over  $R_0^{\text{a}}$  the  $R_2^{\text{a}}$  = constant =  $\beta$   $\gamma$  =  $\gamma$ bary c. Therefore, the number of C molecules at a time  $t + at$  is the<br>time t plus the number gained in association reactions and minus the rate at a contraction. tumber of C molecules at a time  $t + ut$  is the  $\sum_{i=1}^{N} \widetilde{A}^{\mu}$  is defined and signal downstream. The dimerical downstration is unusual.  $\alpha$  and  $\alpha$  at the dissociation reaction processes at the rate beam  $\alpha$ , we have been at the rate beam  $\alpha$ , we have been at the rate beam  $\alpha$  $\mathcal{L}$  $\begin{array}{c}\n\text{mper} \\
\text{ant} = 2 \left[ R \right]_{0} + \frac{2}{2} \left[ R \right]_{0} \\
\end{array}$  $\frac{1}{2}$ in molar units (number of moles of <sup>a</sup> substance per litre). Let [C] denote the molar concentration  $\begin{aligned} \text{because only one} \end{aligned}$ because only one moved in the rate of the concentration for the concentration of the concentration of  $\frac{1}{2}$  in the rate of the rate of the concentration  $\frac{1}{2}$  in the concentration of  $\frac{1}{2}$  in the concentration because only one molecule of dimer**tation**<br>
S.  $\widehat{A}$ *K***ER** identify and then diversion of the association and<br>
s.  $\widehat{A}$  $\widehat{B}$  $\begin{bmatrix} 1, & 1, & 1 \\ 0, & 0, & 0 \\ 0, & 0, & 1 \end{bmatrix}$  of  $\begin{bmatrix} 1, & 0 \\ 0, & 1 \end{bmatrix}$  and  $\begin{bmatrix} 1, & 0 \\ 0, & 1 \end{bmatrix}$  and  $\begin{bmatrix} 1, & 0 \\ 0, & 1 \end{bmatrix}$  and  $\begin{bmatrix} 1, & 0 \\ 0, & 1 \end{bmatrix}$  and  $\begin{bmatrix} 1, & 0 \\ 0, & 1 \end{bmatrix}$  and  $\begin{bmatrix} 1, &$ be molecule of dimer  $\frac{\overbrace{\text{C}_{\text{M}}}\text{Trs}\overbrace{\text{C}_{\text{M}}}\text{Tris}\overbrace{\text{C}_{\text{M}}}\text{Tris}\overbrace{\text{C}_{\text{M}}}\text{Tris}\text{C}_{\text{M}}\text{Tris}\text{C}_{\text{M}}\text{C}_{\text{M}}\text{C}_{\text{M}}\text{C}_{\text{M}}\text{C}_{\text{M}}\text{D}_{\text{M}}\text{D}_{\text{M}}\text{D}_{\text{M}}\text{D}_{\text{M}}\text{D}_{\text{M}}\text{D}_{\text{M}}$ because only one molecule of dimer  $\frac{\sum_{i=1}^n \frac{1}{2} \sum_{i=1}^n \frac{1}{2} \sum_{i=1}$ because only one molecule of dimer**ent and are unchanged and are increased and are increased and are increased and are increased and are unchanged an** Many membrane receptors reversible of three  $\frac{1}{2}$ ,  $\frac{1}{2}$ , because only one molecule of dimer **and**  $G$  with  $\alpha$  because only the section is dimerrican bind  $G$  which is unusual. The moment of pairs of all molecules in the capital downstream. The moment of pairs of and signal pa because only one molecule of dimer  $\frac{\sum_{i} \frac{1}{2} \sum_{j} \frac{1}{2} \sum_{j} \sum_{k} \sum_{k} \frac{1}{2} \sum_{k} \sum_{k} \frac{1}{2} \sum_{k} \sum_{k} \frac{1}{2} \sum_{k} \frac$  $\frac{1}{\text{Res at}}$ mp<br>ant<br>at  $\operatorname*{per}_{\mathbb{C}}\underset{=2}{\operatorname*{pr}}\mathbb{R}$ because only one molecule of dime $\blacksquare$ <br>
Supering a low the number of the multiplactic because the form of the properties of the form of the system of the rate of the rate of the rate of the rate of R are removed by  $\frac{N$ **S Attantive** the for the mumber of reaction and discontinues are released by the released by the released by the molecules are rel and dissociation reactions will be<br>not dissociation reactions will be of pairs of A and B molecules is<br> $r_{R2}^{R1} = \frac{1}{100} \frac{1}{100$ acule:<br>scyle:<br>s s fhe<br>in the i<br>ing<br>ing<br>ing<br>cult<br>cles<br>cult<br>the<br>the cult Taking<br>
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If the output, y, increase The units of the macroscope rate for the matrix of th Taking the limit of  $\hat{d}$  random contains of the number<br>for the number of the maximal different of the maximal of the maximal of the maximal of the pairs of  $\partial_t A_i$  and B molecules<br>e over  $R_i$  the  $T_iA_{i}^L$  = constant Solution that the model of the set of properties in the control of the set of the set of the set of and Reset is an interest of the set of the s Solution is the matrix only the dimerican bind of the matrix of the matrix of the dimerization for the dimerization for the matrix of the matrix of the dimerization reaction is unusually  $\left(\frac{1}{2}\right)$  and  $\left(\frac{1}{2}\right)$  an because only one molecule of duner **Let**<br>
25 denoted by the annual of  $\theta$  condition and Resolution and Resolution sections will be<br>
denoted by the annual of pair of A and B molecule is<br>  $\theta$  ( $\theta$ ) denoted by the reactio  $\mathbb{E}_2[\mathcal{R}]_0^{\sim} + \mathcal{A}$ <br>me  $t + dt$ because only one molecule of dimer $\blacksquare$  set<br>symbol system are rated as the rate of the rate of Arena at distance at the response of the rate of Arena and Because two molecules of R are removed in the removed of R are rem by the molecules of three **line**s  $\frac{1}{2}$ ,  $\frac{1}{2}$ , one molecule of dimer **left** is  $\frac{G}{N}$  and  $\frac{G}{N}$  and  $\frac{G}{N}$  constant in the association and dissociation reactions will be a later). The number of pairs of A and B molecules in a ssociate over  $n_1$  time  $n_2$  a ອີ $\frac{5}{2}$  $\leftarrow$   $\frac{4}{2}$  R<sub>2</sub>les<br>+  $dt$  is the<br>minus the i one molecule of dimer **of hs**  $\overbrace{\mathbb{R}}^{\overbrace{\mathbb{R}}^{\text{reducible}}}_{k}$  is  $\overbrace{\mathbb{R}}^{\text{reducible}}$ . Surfaces  $d\epsilon$ , the association and  $\overbrace{\mathbb{R}}^{\text{reducible}}$   $\overbrace{q}$   $\overbrace{q}^{\text{reducible}}$   $\overbrace{q}^{\text{reducible}}$   $\overbrace{q}^{\text{reducible}}$  and  $\overbrace{q}^{\text{reducible}}$  and because the control of the dimernity of the dimernity of  $\frac{1}{2}$  and  $\frac{1}{2}$  % of dimer $\frac{\delta \hat{\Gamma}}{\delta \hat{\Gamma}}$ ns  $\frac{\hat{\Gamma}^2}{\delta \hat{\Gamma}^2}$  die koliektes. Summing l  $\sum_{\text{odd}}$  on  $\mathcal{B}(\mathcal{L})$  of  $\mathcal{B}(\mathcal{L})$  contains and  $\mathcal{L}(\mathcal{L})$  contains and  $\mathcal{L}(\mathcal{L})$  contains or dimer for dissociation reactions will be  $\mathcal{L}(\mathcal{L})$  and  $\mathcal{L}(\mathcal{L})$  and  $\mathcal{L}(\mathcal{L})$  and  $\mathcal{L}(\$ t a time  $t + d$ stocules the enterpretecules are in the number of pairs of A and B molecules in the dimersion of the dimer,  $R$  molecules the dimersion of  $\alpha$  molecules the dimersion of  $\alpha$  molecules the dimersion of  $\alpha$  molecules the  $\mathbf{U}$ 

If the output,  $y$ , increases with increasing levels of els of input,  $x$ , the appropr  $\lim_{x \to 0} \frac{d}{dx} \left( \frac{d}{dx} \right) = \frac{d}{dx} \frac{d}{dx} \left( \frac{d}{dx} \right) = \frac{d}{dx} \left( \frac{d}{dx} \right) + \frac{d}{dx} \left( \frac{d}{dx} \right)$ <br>
If the output, y, increases with increasing levels of input, x, the appropriate Hill function is  $\mathbf{H}^{\mathbf{F}}$ b[R2] (9) b C[ ]. (7)  $F(t)$  and there there can reach equilibrium, the rate constants cannot be arbitrarily constant be arbitrarily cannot be a reach equilibrium, the rate constants can not be a reach of the rate constants can not be a rate co If the output,  $g$ , increases which increasing levels of input,  $x$ , the appropriate rinf function  $\mathbf{1}$  , and  $\mathbf{3}$  is the volume of the volume of the cell in litres. To  $\mathbf{1}$  is  $\mathbf{1}$  is  $\mathbf{1}$  is a volume of the cell in litres. To volume of the cell in litres. To volume  $\mathbf{1}$  $\theta$  output, y, increa If the output, y, increases with increasing levels of input, x, the appropriate Hill function is  $\alpha$  entrings output,  $y$ , increases with increasing levels of input,  $x$ , the approach  $\alpha$ If the output,  $y$ , increases with increasing levels of input,  $x$ , the appropriate Hi y, increases with increasing  $y(x)$ If the output,  $y,$  increases with increasing levels of input,  $x,$  th If the output  $\mu$  increases with increasing loyels of input  $\pi$  the epprepriate Hill function is If the output, y, increases with increasing levels of input, x, the appropriate Hill function is<br>  $y(x)$   $x^n$  (2.40)  $\mathbb{R}^2$ If the output, y, increases with increasing levels of input,  $x$ , the appropriate Hill function is  $\begin{aligned} \text{If the} \end{aligned}$ interval of time dissociation and dissociation and dissociation reactions will both occur (we will include  $\alpha$ ncreases with increasing levels of input,  $x$ , the appropriate Hill function is dt + 2<br>dt = 0 (10) = 0 (10) = 0 (10) = 0 (10) = 0 (10) = 0 (10) = 0 (10) = 0 (10) = 0 (10) = 0 (10) = 0 (10) = 0 (10 increases w If the output, y, increases with increasing levels of input, x, the appropriate Hill function is If we assume that each of these reactions is at equilibrium and so obeys detailed balance,

If the output, y, increases with increasing levels of input, x, the appropriate Hill function is  
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\frac{d}{dx} \frac{\partial \mathcal{L}}{\partial x} \frac{\partial \mathcal{L}}{\partial y} \frac{\partial \mathcal{L}}{\partial z} \frac
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 $\frac{y(x)}{y_{\text{max}}} = \frac{x^n}{K^n + x^n}$  (2.49)<br>where we call *n* the Hill number, or occasionally the Hill coefficient, and *K* is the value of the<br>input that causes the output to be half of its maximum value ( $y_{\text{max}}$ ). The parame If t<br>
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tion. where we denote the contribution that sometimes of  $\Gamma$  if the out If the output,  $y$ , in<br>where we call  $n$  the E<br>input that causes the<br>sometimes called the  $\overline{\phantom{a}}$ <br>If the output decre<br>and  $K$  is now sometin<br>tion If the output,  $y$ , increases the output that causes the output that causes the output decreases of the output decreases and  $K$  is now sometimes mput that causes the output to be half of its maximum value  $(y_{\text{max}})$ . The parameter K is<br>sometimes called the  $EC_{50}$  of the response, the half-maximal (50%) effective concentration.<br>If the output decreases with increas  $\Box$  get  $\Box$  and  $V$  is the value of the For the output,  $y$ , increasing at the output,  $y$ , increasing where we call  $n$  the Hill n input that causes the output decreases  $\frac{y(x)}{y_{\text{max}}} = \frac{x^n}{K^n + x^n}$  (2.49)<br>where we call *n* the Hill number, or occasionally the Hill coefficient, and *K* is the value of the<br>input that causes the output to be half of its maximum value  $(y_{\text{max}})$ . The paramet 1.  $x$ , the appropriate Hill function is<br>
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constant in the appropriate Hill Chemical rate equations are usually written in terms of concentrations, which are measured If the where vinput the moment of models of the models of the models of the molecular concentration. If the output,  $y$ , in<br>where we call  $n$  the H<br>input that causes the<br>sometimes called the I<br>if the output decre<br>and  $K$  is now sometim<br>tion. the units of the metallical deviation of the matrix of the matrix of the Hill number, or occasionally the Hill coefficies the output to be half of its maximum value of the EC<sub>50</sub> of the response, the half-maximal (50 ut d increasing levels of input, x, the approximate increasing levels of input, x, the approximate  $\frac{y(x)}{y_{\text{max}}} = \frac{x^n}{K^n + x^n}$ <br>r occasionally the Hill coefficient, and e half of its maximum value  $(y_{\text{max}})$ .<br>sponse, the halfthe Hill function is<br>(2.49)<br>is the value of the<br>e parameter  $K$  is For the matrice equation in terms are usually sometimeter in the measured in terms of concentrations, which are measured in terms of concentrations, which are measured in the measured in the measured in the measured in th If the output where we call  $\pi$  input that causes one<br>times calle If the output and  $K$  is now substance the molecular<br>concentration.  $\mathbf v$ where we call *n* the Hill number, or occasionally the Hill coefficient,<br>input that causes the output to be half of its maximum value  $(y_m)$ <br>sometimes called the EC<sub>50</sub> of the response, the half-maximal (50%) e 1.1.1 Example: dimerization where we call *n* the Hill number, or oo input that causes the output to be h<br>sometimes called the  $EC_{50}$  of the responent increases with increases<br>If the output decreases with increases<br>and *K* is now sometimes called t is the value of the<br>e parameter  $K$  is<br>concentration.<br>te Hill function is<br> $(2.50)$ <br>ubitory concentrawhere viright to input the sometime in the sometime in the solution in the same of  $K$  tion. where we call  $n$ <br>input that causes<br>sometimes calle<br>If the output<br>and  $K$  is now substance the molecular concentration. sometim<br>If th mpat in<br>sometim<br>If th where we call *n* 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  $(y_{\text{max}})$ . The parameter *K* is sometimes called the EC<sub>50</sub> Function is<br>  $(2.49)$ <br>
alue of the eter  $K$  is<br>
at the ration sometimes called the  $EC_{50}$  of the response, the half-maximal  $(50\%)$  effective concentration.<br>If the output decreases with increasing levels of input, then the appropriate Hill function is associ<br>
where we call *n* the Hill number<br>
If the output, *y*, increases<br>
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If the out If the output,  $y$ , incompared in the cell  $n$  the Hilbert causes the sometimes called the E<br>If the output decreases.  $\overline{\mathbf{r}}$ while mumber of pairs of pairs of  $\frac{1}{2}$  and  $\frac{1}{2}$  is equalled. The number of  $\frac{1}{2}$  and  $\frac{1}{2}$  where we call *n* the Hill number, or occasionally the Hill coefficient, and *K* is the value of the<br>input that causes the output to be half of its maximum value  $(y_{\text{max}})$ . The parameter *K* is<br>sometimes called the EC<sub>50</sub>  $r_{\text{wk}}$ <br>
a<br>  $r_{\text{wk}}$ <br>  $\text{e}$ <br>  $\text{u}$ <br>  $\text{u$ B molecules<br>
of C molecule<br>  $R_0^F - 2[R_2]_0^F$ <br>  $e^t + dt$  is the<br>
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coefficient, and K is the value of the<br>
coefficient, and K is the va  $\begin{minipage}[c]{0.7\linewidth} \centering \begin{tabular}{l} \multicolumn{2}{c}{\textbf{I}} \end{tabular} \end{minipage} \begin{minipage}[c]{0.7\linewidth} \centering \begin{tabular}{l} \multicolumn{2}{c}{\textbf{I}} \end{tabular} \end{minipage} \end{minipage} \begin{minipage}[c]{0.7\linewidth} \centering \begin{tabular}{l} \multicolumn{2}{c}{\textbf{I}} \end{tabular} \end{minipage} \end{minipage} \begin{minipage}[c]{0.7\linewidth} \centering \begin{tabular}{l} \multicolumn{2}{c}{\textbf{I}} \end{$ If the output,  $y$ ,<br>where we call  $n$  the<br>input that causes the sometimes called the<br>If the output dec<br>and  $K$  is now somet<br>tion. where  $\omega$  over  $R_1$  imerged. The number of  $G$  and  $B$  mode over  $R_1$  imerged. The number of  $G$  is  $\mathbb{R}[G]$  =  $\mathbb{R}[G]$  =  $\mathbb{R}[G]$  =  $\mathbb{R}[G]$  =  $\mathbb{R}[G]$  =  $\mathbb{R}[G]$  +  $\mathbb{R}[G]$  +  $\mathbb{R}[G]$  +  $\mathbb{R}[G]$  + of its many<br>e, the hall<br>g levels of between<br>
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ient, and K is the value of the<br>  $(y_{\text{max}})$ . The parameter K is<br>
%) effective concentration.<br>
the appropriate Hill % e Hill function is<br>
(2.49)<br>  $\colon$  the value of the<br>
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oncentration.<br>
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int, and K is the value of the<br>  $(y_{\text{max}})$ . The parameter K is<br>
(b) effective concentration.<br>
e appropriate Hill function is<br>
(2.50)<br>
naximal inhibitory concentrawhere we call *n* 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  $(y_{\text{max}})$ . The parameter *K* is sometimes called the EC<sub>50</sub> If the output, y, increases with incompare we call n the Hill number, or comput that causes the output to be l sometimes called the EC<sub>50</sub> of the responding in the output decreases with increases with increases with incre where we call *n* the Hill number, or occasionally the Hill coefficient, and *K* is the value of the<br>input that causes the output to be half of its maximum value  $(u_{\text{max}})$ . The parameter *K* is the output, y, increases with increasing levels of input, x, the appropriate Hil<br>  $\frac{y(x)}{y_{\text{max}}} = \frac{x^n}{K^n + x^n}$ <br>
we call n the Hill number, or occasionally the Hill coefficient, and K is the chat causes the output to be ha sometimes called the  $EC_{50}$  of the response, the half-maximal (50%) effective concentration.<br>If the output decreases with increasing levels of input, then the appropriate Hill function is (2.49)<br>the value of the<br>parameter  $K$  is<br>pncentration.<br>PHIII function is<br>(2.50)<br>bitory concentrawhere input<br>some Iff some<br>and in terms of concentrations, where  $\frac{1}{2}$ <br>and  $\frac{1}{2}$ where we call input that cases<br>in that cases of the output of the output of the output<br>and  $K$  is now tion. where<br>
input<br>
somet<br>
If the outp<br>he  $EC_{50}$ <br>ecreases  $\frac{x^n}{n+x^n}$  (2.49)<br>the Hill coefficient, and K is the value of the<br>naximum value ( $y_{\text{max}}$ ). The parameter K is<br>alf-maximal (50%) effective concentration.<br>of input, then the appropriate Hill function is<br> $\frac{K^n}{n+x^n}$  (2.5 sometimes called the  $EC_{50}$  of the response, the half-maximal (50%) effective concentration.<br>If the output decreases with increasing levels of input, then the appropriate Hill function is  $y_{\text{max}}$   $K^n + x^n$ <br> *K* is the value the Hill coefficient, and *K* is the value The units of the matrices of the matrices rate f and the matrices rate f and the matrices rate f and the matrices rate f are M1 state  $\frac{1}{2}$  state  $\frac{1}{2}$  state  $\frac{1}{2}$  state  $\frac{1}{2}$  state  $\frac{1}{2}$  state  $\frac{1}{2}$ 1.1.1 Example: dimerization blevide<br>  $\hat{t}$  is the<br>  $\hat{t}$  is the<br>  $\hat{t}$  is the<br>
blevide<br>  $\hat{R}$  is the<br>  $\hat{R}$  and  $K$  is the value of the<br>
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\text{mF$ reached the correction is<br>reaction is<br> $(2.50)$ <br>necentra-Finally function is<br>(2.49)<br>is the value of the<br>ne parameter  $K$  is If the output,  $y$ , in<br>
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input that causes the<br>
sometimes called the I<br>
If the output decre<br>
and  $K$  is now sometim the output,  $y$ , increased by the Hill  $\frac{1}{2}$ reactions rate of  $\mathcal{E}[K_1]$  and  $\mathcal{E}[K_2]$  are constant  $=2|\mathbf{K}|_0$ <br>tput, *y*, increases with increasing levels of input, *x*,<br> $\frac{y(x)}{y_{\text{max}}} = \frac{x^n}{K^n + x^n}$ <br>all *n* the Hill number, or occasionally the Hill coeff<br>cau If number, or occasionally the Hill coefficient, and K is the value of the output to be half of its maximum value  $(\ell_{\text{max}})$ . The parameter K is bet, of occasionally the fifth coefficient, and  $K$  is the value of the to be half of its maximum value  $(y_{\text{max}})$ . The parameter  $K$  is the response, the half-maximal (50%) effective concentration.<br>The increasing levels 1.1.1 Example: dimerization e Hill number, or occasionally the output to be half of its m<br>he EC<sub>50</sub> of the response, the ha<br>ecreases with increasing levels c<br> $\frac{y(x)}{y_{\text{max}}} = \frac{f}{K^r}$ <br>etimes called the IC<sub>50</sub> of the resp m is<br>  $(49)$ <br>
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e value of the rameter  $K$  is<br>
entration.<br>
ill function is imes called the  $EC_{50}$  of the response, the half-maximal  $(50\%)$  effective concentration.<br>the output decreases with increasing layels of input, then the eppreciate Hill function is out put to be frain of its maximum value  $(y_{\text{max}})$ . The parameters

<span id="page-12-0"></span>2.1 The Hill number output decreases with increasing levels of input, then the approprate Hill function is  $\frac{1}{2}$  $\epsilon_{50}$  or the response, the nan-maximal (50%) enective concentration.<br>Ses with increasing levels of input, then the appropriate Hill function.  $\frac{1}{\sqrt{2}}$ 

sometimes called the EC<sub>50</sub> of the response, the half-maximal (50%) effective concentration.  
\nIf the output decreases with increasing levels of input, then the appropriate Hill function is  
\n
$$
\frac{y(x)}{y_{\text{max}}} = \frac{K^n}{K^n + x^n}
$$
\nand K is now sometimes called the IC<sub>50</sub> of the response, the half-maximal inhibitory concentration. (2.50)

 $\overline{\phantom{a}}$ w sometimes called the  $\rm IC_{50}$  of the response, the half-maximal inhibitory concentrations of the response, the half-maximal inhibitory concentrations, and  $\lim$ .  $\lim_{n \to \infty}$  is the concentration  $B$ naximal  $y_{\text{max}}$   $K^{\alpha} + x^{\alpha}$ <br>d the IC<sub>50</sub> of the response, the half and  $K$  is now sometimes called the  $IC_{50}$  of the response, the half-maximal inhibitory con dt <sup>=</sup> ˜<sup>f</sup> <sup>n</sup><sup>A</sup><sup>V</sup> <sup>A</sup>[ ][<sup>B</sup> % es called the  $\rm IC_5$ the output to be half of its r<br>
e EC<sub>50</sub> of the response, the half reases with increasing levels<br>  $\frac{y(x)}{y_{\text{max}}} = \frac{1}{K}$ <br>
imes called the IC<sub>50</sub> of the res  $V_{\text{MOM}}$ and K is now sometimes called the  $IC_{50}$  of the response, the half-maximal inhibitory concentration.  $rac{y(x)}{y_{\text{max}}} = \frac{K}{K^n + x^n}$  (2.50)<br>and K is now sometimes called the IC<sub>50</sub> of the response, the half-maximal inhibitory concentra-<br>tion. and  $K$  is now sometimes<br>tion.  $\overline{R}$ in molecular units (number of molecular concentration). Let  $\mathcal{L}$  denote the molecular concentration of molecular concentration of  $\mathcal{L}$ and  $K$  is now sometimes called the  $IC_{50}$  of the response, the half-maximal inhibitory concentration and K is now sometimes called the  $IC_{50}$  of the resp<br>tion  $\frac{1}{x}$  +  $\frac{1}{x}$  (34)  $\frac{1}{x}$  +  $\frac$ and K is now sometimes called the  $IC_{50}$  of the response, the half-maximal inhibitory concentra- $\mathsf{P}$ he half-max and K is now sometimes called the  $IC_{50}$  of the response, the half-maximal inhibitory concentra- $F_{\rm tot}$  and  $F_{\rm tot}$  rate can reach equilibrium, the rate constants cannot be arbitrarily cannot be a rate constant of  $F_{\rm tot}$  $\sqrt[n]{\frac{n+x^n}{n}}$ <br>sponse, the ha  $\frac{1}{\sqrt{2}}$  into an eq. 3 into an eq. n in the concentral entries van Eq. 3 by  $\alpha$ 



#### <span id="page-13-0"></span>2.7 Describing response curves

The Hill number is often used to characterise the ultrasensitivity of the response. From the definition of the Hill function, its sensitivity at  $x = K$  is

<span id="page-13-4"></span>
$$
\left. \frac{d \log y / y_{\text{max}}}{d \log x} \right|_{x = K} = \frac{n}{2} \tag{2.51}
$$

and is determined solely by the Hill number. 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. [2.89,](#page-22-0) 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\)](#page-37-0).

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 and are often called cooperative for systems involved in gene regulation. A response with a Hill number below one is sub-sensitive.

#### <span id="page-13-1"></span>2.7.1 Sensitivity

With sensitivity analysis, we aim to determine how the behaviour of a model depends on its parameter values. The local sensitivity of a steady-state quantity s with respect to changes in a parameter p is  $ds/dp$ . A small change in p, denoted  $\Delta p$ , gives

$$
s(p + \Delta p) \simeq s(p) + \frac{ds}{dp}\Delta p + \mathcal{O}\left(\Delta p^2\right)
$$
\n(2.52)

from a Taylor expansion, or

<span id="page-13-2"></span>
$$
\Delta s = s(p + \Delta p) - s(p) \simeq \frac{ds}{dp} \Delta p. \tag{2.53}
$$

The local sensitivity therefore measures how a small  $\Delta p$  affects s. It is, however, unhelpful for comparing different sensitivities because it has units: the units of  $s$  divided by the units of  $p$ . Different sensitivities may have different units.

The relative local sensitivity is more common; it is dimensionless. We define the relative sensitivity as

<span id="page-13-3"></span>
$$
\chi = \frac{ds/s}{dp/p} = \frac{p}{s} \times \frac{ds}{dp} = \frac{d \log s}{d \log p}.
$$
\n(2.54)

From Eq. [2.53,](#page-13-2)

$$
\frac{\Delta s}{s} \simeq \frac{1}{s} \frac{ds}{dp} \times \Delta p
$$

$$
= \frac{p}{s} \frac{ds}{dp} \times \frac{\Delta p}{p}
$$

and so, from Eq. [2.54,](#page-13-3) the relative sensitivity satisfies

$$
\frac{\Delta s}{s} \simeq \chi \frac{\Delta p}{p}.\tag{2.55}
$$

The relative sensitivity therefore measures the fractional change in s resulting from a small fractional change in p. Fractional changes are absolute and so make clear what we mean by 'small'.

The Hill number measures the relative sensitivity of the output  $y$  to a change in the input x when the input is at the threshold value,  $x = K$ . The Hill number, n, is twice the relative sensitivity from Eq. [2.51:](#page-13-4)

<span id="page-14-2"></span>
$$
n = 2 \left. \frac{d \log y}{d \log x} \right|_{x=K} \tag{2.56}
$$

Eq. [2.56](#page-14-2) implies that a system with a high sensitivity at the threshold level of input will be a sharp, ultrasensitive switch with a high Hill number. For such systems, a small fractional change in input can cause a large factional change in output, such as when the input crosses the threshold level.

#### <span id="page-14-0"></span>2.8 Modelling signal transduction II

Considering Fig. [1,](#page-2-1) we can use a Hill function to immediately write an equivalent to Eq. [2.38:](#page-9-2)

$$
[R^*] \simeq \frac{R_0 [S]^n}{K^n + [S]^n} \tag{2.57}
$$

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. [2.40](#page-9-1) then becomes:

<span id="page-14-3"></span>
$$
\frac{d[A^*]}{dt} \simeq \frac{kR_0[S]^n}{K^n + [S]^n} (A_0 - [A^*]).
$$
\n(2.58)

# <span id="page-14-1"></span>2.9 Allostery – and the Monod-Wyman-Changeux model – as a means to generate ultrasensitivity

An enzyme is allosteric if its activity is modified by a regulator binding to a site on the enzyme that is not the enzyme's functional site. Binding sites on allosteric enzymes interact through conformational changes: a molecule binding at a regulatory site causes a change in conformation at the active site and so alters enzymatic activity.

Allostery explains why a molecule that regulates an enzyme need not have a similar structure to the enzyme's substrate. This freedom in the structure of regulatory molecules was a great revelation when discovered in the 1950s.

As an example consider a membrane receptor that activates when bound by an extracellular ligand. Biochemically the receptor has two conformational states: one active and one inactive. The active state can signal downstream; the inactivate state cannot. An extracellular ligand activates the receptor by preferentially binding to the active state over the inactive one. Through this preference, the ligand stabilises the receptor in the active state. Once active, the receptor may bind a signalling molecule on its cytoplasmic side. This signalling molecule can have a completely different structure from the ligand because the ligand binds to a different site on the receptor.

Allostery is one way to generate ultrasensitive responses, and a celebrated model is the concerted model of Monod, Wyman, and Changeux [\[6\]](#page-63-5). 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 consider the<br>enzyme to be 'on' with high activity: in the relaxed state, it is 'off' with low activity. Any enzyme to be 'on' with high activity; in the relaxed state, it is 'off' with low activity. Any molecule that has a higher binding energy for the  $T$  state relative to the  $R$  state activates the molecule that has a higher binding energy for the  $T$  state relative to the  $R$  state activates the enzyme. enzyme. molecule 1<br>enzyme. - and spontan<br>
enzyme to be<br>
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enzyme to be 'on' wit<br>
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enzyme to be 'on' with high active<br>
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enzyme.<br>
2.9.1 Allosteric molecules with – and spontaneously changes between these conformations. In the tense s<br>enzyme to be 'on' with high activity; in the relaxed state, it is 'off' wit<br>molecule that has a higher binding energy for the  $T$  state relative to t F' with low activity. Any<br>  $\mathbf{F}$  is the R state activates the<br> **egulator**  $\alpha$  = and spontaneously changes between these conformations. In the tense state, we consider the chang ith his injeture included in the random collection of the contractive system  $L =$  active  $J$ recust  $\sum_{i=1}^{\infty}$  as a set of a set o spont<br>ule the discourse at a reaction reaction reaction reaction reaction reaction reaction process at the rate of chang ith higher<br>
nolect<br>
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reversibly dimersion<br>  $L =$ <br>
activat<br>  $f$ reaction. Although the association. Although the association of the as consid<br>tivity.<br>ctivat<br>-<br>crde: - and spontaneously changes between these conformations. In the tense state, we consider the enzyme to be 'on' with high activity; in the relaxed state, it is 'off' with low activity. Any molecule that has a higher bindin chang ith hi<br>nigher<br>nolecules for the rate of the rate of the rate of the rate of<br> $L =$ <br>activa  $f$ requires the association of  $\frac{1}{2}$  and  $\frac{1}{2}$  are the association of  $\frac{1$ spont to the discount of the discociation  $L$  is the disconresponding at the fraction  $\mathbf{R}$  the fraction  $\math$ chang ith higher<br>
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f reaction. Although the association of the associati consid<br>tivity.<br>ctivat<br>ion reaction<br>the endertiancy The rate of the system are at  $L =$  activa  $J$  are removed  $\begin{align} \text{recons} \text{ } \text{and} \text{ } \text{the} \text{ } \text{.} \text{ } \text{therefore} \end{align}$ spont to ule the discourse to differ a reached at a reached the discontinuity of  $L$  is the fraction of the fraction  $\mathbf{r}$  the fra chang ith higher<br>
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# 2.9.1 Allosteric molecules with a single binding site for a regulator  $\frac{1}{2}$  a regulator<br> $\frac{1}{2}$ 2.9.1 Allost 2.9.1 Allosteric molecules with a single binding site for a regulator



Let a regulatory molecule be  $X$  — shown as a red triangle. In the Let a regulatory molecule be  $X$  — shown as a describe the spontaneous conformational changes as<br> $T \stackrel{L}{\Longleftarrow} R$ angle. In describe the spontaneous conformational changes as  $T \stackrel{L}{\Longrightarrow} R$ reaction proceeds at the rate of  $\frac{1}{R}$ Let a regulatory molecule be  $X$  — shown as a red triangle. In the absence of  $X$ , we can cribe the spontaneous conformational changes as

<span id="page-15-0"></span>
$$
T \xrightarrow{L} R
$$

 $T \rightleftharpoons R$ where L is the equilibrium constant:  $L = [R]/[T]$ . The binding reactions are second-order:<br> $X + T \frac{K_T}{T} T$  $\overline{\text{a}}$  is the equili b  $L =$  $[D]/[T]$  Th he bi  $[D]/[T]$  The bindin where L is the equilibrium constant:  $L = [R]/[T]$ . The binding reactions are second-order:

$$
X + T \xleftarrow{K_T} T_1
$$
 and  

$$
X + R \xrightarrow{K_R} R
$$

and  $\mathbf{r}$ and

$$
\mathbf{X}+\mathbf{R}\xrightarrow{K_{R}}\mathbf{R}_{1}
$$

 $\frac{d\mathbf{r}}{d\mathbf{r}}$ vided by the rate of the dissociation reaction. Let  $K_T > K_R$  so tha<br>cause it favours binding the 'on' state T.  $X + T \xleftarrow{K_T} T_1$ <br>and  $X + R \xleftarrow{K_R} R_1$ <br>with  $K_T$  and  $K_R$  being association equilibrium constants – the rate of the association reaction because it favours binding the 'on' state  $T$ .<br>
If there is only one hinding site for  $Y$  on the engrise and essume with  $K_T$  and  $K_R$  being association equilibrium constants – the rate of the association reaction<br>divided by the rate of the dissociation reaction. Let  $K_T > K_R$  so that X activates the enzyme<br>because it favours binding the divided<br>because<br>If the  $r_{\rm{short}}$ <br>is equidently the rate ours discussed by the rate ours discussed by the rate ours discussed by the relation of  $\alpha$  $\mathbf{r}$ and K<br>i favou<br>re is c<br>m:<br>fraction  $\frac{1}{\sqrt{2}}$  $X + R \stackrel{K_R}{\Longleftarrow} R_1$  with  $K_T$  and  $K_T$  being association equilibrium constants – the rate of the association reaction. divide<br>becau 2 and by the rate of the dissociation reaction. Let  $K_T > K_R$  so that X activates the enzyme<br>se it favours binding the 'on' state T.<br>there is only one binding site for X on the enzyme and essuming each reaction is at reaction and two modes are required by  $K_R$  be rate relevant on  $\mathfrak c$  is only the relation of  $\mathfrak c$ quilibrium constants<br>
i reaction. Let  $K_T$ <br>
ite T.<br>
for X on the enzy:<br>  $| = K_T[T][X]$  ; [A reaction and two modes are released by the rate relation contains a relation of  $\mathcal{L}$  and  $\mathcal{L}$  are relation of  $\mathcal{L}$ divided by the rate of the dissociation reaction. Let  $K_T > K_R$  so that X activates the enzyme

dia amin'ny fivondronan-kaominin'i Castro<br>Jeografia  $L=$ reactive intervalse it for the rate of the rate of  $\mathbf{r}$  and  $\mathbf{r}$  are the fraction of  $\math$ If there is only one binding site for  $X$  on the enzyme and assuming each reaction is at equilibrium:

$$
L = \frac{[R]}{[T]} \quad ; \quad [T_1] = K_T[T][X] \quad ; \quad [R_1] = K_R[R][X]. \tag{2.59}
$$
\nThen the fraction of activated enzymes is

\n
$$
[T] \cup [T_1]
$$

<span id="page-15-1"></span> $\frac{d}{dx}$ 

and spontaneously changes between these conformations. In the tensor state, we consider the  
enywe be to be 'with high activity in the relevant state, it is of with low activity. Any  
measurable that has a higher binding energy for the *T* state relative to the *R* state activities the  
energy. **2.9.1 Allosteric molecules with a single binding site for a regulator**  
**2.9.1 Allosteric molecules with a single binding site for a regulator**  
**2.9.1 Allosteric molecules with a single binding site for a request**  
**2.9.2**  
**2.9.2**  
**2.9.1 Allosteric molecules with a single binding site for a request**  
**2.9.2**  
**2.9.1 Allosteric molecules with a single binding site** for a regularity of *X*, we can  
describe the spontaneous conformationed changes as  

$$
T \xrightarrow{K_T} T
$$
  
**2.1**  
**2.1**  
**2.2**  
**3.1**  
**3.1**  
**4.1**  
**5.1**  
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**8.1**  
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**11**  
**11**  
**12**  
**13.1**  
**14**  
**15.1**  
**16**  
**17.1**  
**18.1**  
**1**

which is a hyperbolic function increasing with  $[X]$ . The enzymatic activity increases with X because X biases the molecule to adopt the active conformation which is a hyperbond function increasing with  $[X]$ . The enzymatic absention because X biases the molecule to adopt the active conformation. form a C molecule; the second occurs when a C molecule dissociates back into an A and a B which is<br>because % activity increases with  $X$ with  $[X]$  $\frac{1}{2}$ h<br>eti  $\frac{1}{2}$ asing<br>ppt\_ti<br>woon the active conformation  $\frac{1}{2}$  The<br> $\frac{1}{2}$ which is a hyperbolic function increasing with  $[X]$ . The enzymatic activity increases with  $X$ 

Allowing a two-way reaction between  $T_1$  and  $R_1$  would not change Eq. 2.60. This reaction Allowing a two-way reaction between  $T_1$  and  $R_1$  would not change Eq. 2.60. This reaction<br>would not prevent the others from reaching equilibrium, and its equilibrium constant,  $L_1$  say, must obey  $L_1 = \frac{K_R}{K_T}$ must obey  $L_1 = \frac{K_R}{K_T} L$  because then the set of reactions form a thermodynamic cycle (Sec. 2.5). R2 which is a hyperbolic<br>because X biases the<br>Allowing a two-w<br>would not prevent the<br>must obey  $L_1 = \frac{K_R}{K_T} L$ use X biases the molec<br>
llowing a two-way read<br>
l not prevent the other<br>
obey  $L_1 = \frac{K_R}{K_T} L$  becau<br> **Allosteric molecu** with  $[X]$ . The enz<br>he active conformat<br> $T_1$  and  $R_1$  would no<br>ng equilibrium, and<br>t of reactions form a w[o](#page-10-0)uld not prevent the others from reaching equilibrium, and its equilibrium constant,  $L_1$  say,<br>must obey  $L_1 = \frac{K_R}{K_T} L$  because then the set of reactions form a thermodynamic cycle (Sec. 2.5).<br>2.9.2 Allosteric molecul ch is a<br>ause *1*<br>Allowind not<br>st obey<br>**2** A and the discontinuity reaction  $L_1$  (Sec. 2)  $b_1 = \frac{K_R}{K_T}L$  because then the set of reactions form a thermodynamic cyc hyperboiases<br>g a two<br> $r$ even<br> $L_1 = \frac{I}{I}$  and the dissociationreaction proceeds at the rate increaction.<br>1. Although the association.<br>2. Although the association. and the discontinuity reaction  $L_1$ <br>(Sec. 2) hyperboiases<br>g a two<br>preven<br> $L_1 = \frac{P}{I}$ osteri ch is a<br>
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## 2.9.2 Allosteric molecules with two binding sites for regulators losteric molecules with two binding sites for regulators 2.9.2 Allosteric molecules with two binding sites for regulators.<br> $\mathbf{r}$ must obey  $L_1 = \frac{1}{K_T}L$  because their the set of reactions form a thermodynamic cycle (Sec. 2.5).<br>2.9.2 Allosteric molecules with two binding sites for regulators



changes. If the enzyme has two identical binding sites for X and if  $f<sub>T</sub>$  is the rate of binding one of those sites then the association reaction becomes  $v_{tr}$ changes. If the enzyme has two identical binding sites for  $\Lambda$  and if  $f_T$  is to of those sites then the association reaction becomes Allc<br>changes  $R_1 = R_1 + R_2$  $R_2 \rightarrow R_2 \rightarrow R_2 \rightarrow R_1$ <br>y can give sharp, switch-like responses as the correction has two identical binding sites for g sites for X and if  $f_T$  is the rate of binding one<br>mes<br> $\frac{2f_T}{\sqrt{T}}$  T<sub>1</sub> Allostery can give sharp, switch-like responses as the concentration of the regulatory molecule

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

because there are now two choices of binding site for  $\Lambda$ . Denoting the  $b_T$ , the dissociation reaction remains  $T_1 \xrightarrow{b_T} X + T$  $b_T$ , the dissociation reaction remains because there are now two choices of  $b_T$ , the dissociation reaction remains because there are now two choices of binding site for X. Denoting the rate of dissociation by  $b_T$ , the dissociation reaction remains  $\Gamma_1$ , one dimer forms or dimer forms or dimer forms or  $\Gamma_1 \xrightarrow{b_T} X + T$  $\label{eq:1} \text{X}+\text{T} \overset{2j}{\rightharpoonup}$  w two choices of binding sit <sup>2</sup> + 2b[R2] (8) frame there are now two choices of binding site for X. Denoting the rate of dissociation<br>the dissociation reaction remains because there are now two choices of binding site for  $X$ . Denoting the rate of dissociation by

<span id="page-16-0"></span>
$$
T_1 \xrightarrow{b_T} X + T
$$

because there is only one way that X can dissociate from  $T_1$ . The ove  $p_T$ , the dissociation<br>because there is<br>is therefore  $2f_T$  /  $T_1 \longrightarrow X + T$ <br>because there is only one way that X can dissociate from  $T_1$ . The overall association constant  $T_1 \xrightarrow{b_T} X + T$ <br>because there is only one way that Y can dissociate from  $T$ . The evenll essesiation constant because there is only one way that X can dissociate from  $T_1$ . The overall association constant<br>is therefore  $2f_T/b_T = 2K_T$ .<br>We can understand this factor of two by considering explicitly the enzyme's two binding sites<br>for is therefore  $2f_T/b_T = 2K_T$ .

We can understand this factor of two by considering explicitly the enzyme s two binding stes<br>for X. Let  $T_{0,0}$  denote an enzyme in the tense state with no bound X molecules;  $T_{1,0}$  denote<br>a tense enzyme with an X boun which is a hypotenuse X bi<br>
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to yought to the Allostery can give sharp, switch-like responses as the concentration of the regular<br>changes. If the enzyme has two identical bunding site for X and if  $f_r$  is the rate of those sites then the association reaction becomes<br> bound to the second site. Then the reactions are<br>  $X + T_{0,0} =$ <br>
and<br>  $X + T_{0,0} =$ note an enzyme in the<br>  $h$  an  $X$  bound to the<br>  $d$  site. Then the react:<br>  $Y$ tion of the regulatory molecule<br>if  $f_T$  is the rate of binding one<br>ing the rate of dissociation by<br>me overall association constant<br>the enzyme's two binding sites<br>und X molecules;  $T_{1,0}$  denote<br>te a tense enzyme with an becomes as the concentration of the regulatory molecule<br>nding sites for X and if  $f_T$  is the rate of binding one<br>becomes<br> $+ T \frac{2f_T}{T} \sum T_1$ <br>ing site for X. Denoting the rate of dissociation by<br> $\frac{b_T}{T} \sum X + T$ <br>dissociate f We can understand this factor of two by considering explicitly the enzyme's two binding sites X. Let  $T_{0,0}$  denote an enzyme in the tense state with no bound X molecules;  $T_{1,0}$  denote ise state with no bound X molecules;  $T_{1,0}$  denote<br>t site; and  $T_{0,1}$  denote a tense enzyme with an X<br>are<br> $f_T$ , activi<br>ge Eq.<br>iilibriu<br>odyna<br>ators<br>ators<br>werall<br>a rense<br>atense atory i<br>
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reactions are  
\n
$$
X + T_{0,0} \frac{f_T}{\frac{f_T}{b_T}} T_{1,0}
$$
\n
$$
X + T_{0,0} \frac{f_T}{\frac{f_T}{b_T}} T_{0,1}
$$
\n17

and

and  
\n
$$
X + T_{0,0} \frac{f_T}{b_T} T_{0,1}
$$
  
\n $T_{0,1}$   
\n17

We can write down the differential equation for  $X$ , which will have four terms because of the four reactions:  $\mathbf{d}$ [ $\mathbf{x}$ z]

<span id="page-17-0"></span>
$$
\frac{d[X]}{dt} = -f_T[X][T_{0,0}] - f_T[X][T_{0,0}] + b_T[T_{1,0}] + b_T[T_{0,1}]
$$
\n
$$
= -2f_T[X][T_{0,0}] + b_T[T_{1,0}] + b_T[T_{0,1}]
$$
\n(2.61)

If we define  $[T_1] = [T_{1,0}] + [T_{0,1}]$  to be the concentration of the enzyme with one molecule of X bound irrespective of where that molecules binds, then Eq. [2.61](#page-17-0) becomes

$$
\frac{d[X]}{dt} = -2f_T[X][T_{0,0}] + b_T[T_1]
$$
\n(2.62)

which is the differential equation that describes the reaction

$$
X+T \xrightarrow[t]{2f_T} T_1
$$

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

For the binding of a second  $X$ , we have

$$
X + T_1 \xrightarrow{f_T} T_2
$$

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. So

$$
T_2 \xrightarrow{2b_T} X + T_1
$$

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:

$$
L = \frac{[R]}{[T]} \; ; \; [T_1] = 2K_T[T][X] \; ; \; [T_2] = \frac{K_T}{2}[T_1][X] \; ; \; [R_1] = 2K_R[R][X] \; ; \; [R_2] = \frac{K_R}{2}[R_1][X] \; (2.63)
$$

Then the fraction of activated enzymes is

$$
f_{\text{on}} = \frac{[T] + [T_1] + [T_2]}{[T] + [T_1] + [T_2] + [R] + [R_1] + [R_2]}
$$
  
\n
$$
= \frac{[T] + 2K_T[X][T] + \frac{1}{2}K_T[X]2K_T[X][T]}{[T] + 2K_T[X][T] + \frac{1}{2}K_T[X]2K_T[X][T] + L[T] + 2K_R[X]L[T] + \frac{1}{2}K_R[X]2K_R[X]L[T]} \\
= \frac{1 + 2K_T[X] + K_T^2[X]^2}{1 + 2K_T[X] + K_T^2[X]^2 + L(1 + 2K_R[X] + K_R^2[X]^2)} \\
= \frac{(1 + K_T[X])^2}{(1 + K_T[X])^2 + L(1 + K_R[X])^2}
$$
\n(2.64)

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

<span id="page-18-1"></span>

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  $K_R$ .

#### <span id="page-18-0"></span>2.9.3 Allosteric molecules with n binding sites for regulators

For  $n$  binding sites,

<span id="page-18-2"></span>
$$
f_{\text{on}} = \frac{(1 + K_T[X])^n}{(1 + K_T[X])^n + L(1 + K_R[X])^n}
$$
\n(2.65)

and the sharpness of the switch increases, with a maximum Hill number of n (Fig. [2\)](#page-18-1). If  $[X_{50}]$ is the concentration of X that makes  $f_{\text{on}}$  half maximal, so that  $f_{\text{on}} = \frac{1}{2}$  $\frac{1}{2}$ , then Eq. [2.65](#page-18-2) implies

$$
L\left(1+K_R\left[X_{50}\right]\right)^n = \left(1+K_T\left[X_{50}\right]\right)^n. \tag{2.66}
$$

Multiplying by [T] and using  $[R] = L[T]$ , we have that

$$
[R] (1 + K_R [X_{50}])^n = [T] (1 + K_T [X_{50}])^n
$$
\n(2.67)

showing that the total concentrations of relaxed and tense allosteric molecules are equal at  $[X] = [X_{50}],$  as expected.

Considering the behaviour near  $[X] = [X_{50}]$  helps build intuition on why the response be-comes more sigmoidal for larger n. By differentiating Eq. [2.65,](#page-18-2) we can show that the rate at which  $f_{\text{on}}$  changes with  $[X]$  is proportional to n:

<span id="page-18-3"></span>
$$
\frac{\partial f_{\text{on}}}{\partial [X]} \bigg|_{[X_{50}]} = \frac{n}{4} \cdot \frac{K_T - K_R}{(1 + K_R [X_{50}]) (1 + K_T [X_{50}])}.
$$
\n(2.68)

This gradient increases with increasing [X] if X binds preferentially to the T states,  $K_T > K_R$ ; the gradient decreases with increasing [X] if X binds preferentially to the R state,  $K_T < K_R$ . When  $[X] = [X_{50}]$ , a small increase in the concentration of X will shift the equilibrium for each of X's binding reactions towards having more X bound. For an allosteric molecule with  $n$ binding sites for X, all n reactions will shift, with those involving tense molecules increasing  $f_{\text{on}}$ by an amount proportional to  $nK_T$ , and those involving relaxed molecules decreasing  $f_{\text{on}}$  by an amount proportional to  $nK_R$ , giving the positive and negative terms in Eq. [2.68.](#page-18-3)

With more than one binding site for the regulatory molecule, the Monod, Wyman, and Changeux model assumes that all the enzyme's binding sites transition together  $-$  in a concerted manner – between the two conformational states  $[6]$ . All binding sites on the enzyme are therefore always in the same conformational state. Typically these binding sites are on identical subunits, and we consider each subunit to have the same conformation. Other models of allostery relax the concerted assumption [\[7\]](#page-63-6).

#### <span id="page-19-0"></span>2.9.4 Limits of the Monod-Wyman-Changeux equation

We can build intuition about Eq. [2.65](#page-18-2) by considering various limits.

• Eq. [2.65](#page-18-2) always includes basal levels of activation. If there are no input molecules present

$$
f_{\text{on}}([X] = 0) = \frac{1}{1 + L} \tag{2.69}
$$

and there is basal activation providing  $L \ll 1$ . Remember that  $|T| = |R|/L$ , and so  $L \ll 1$ implies that molecules are often 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 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. [2.65](#page-18-2) becomes

$$
f_{\text{on}}(K_R[X] \gg 1) \simeq \frac{K_T^n}{K_T^n + L K_R^n} \tag{2.70}
$$

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

$$
f_{\rm on} \simeq \frac{c^n}{c^n + L} \tag{2.71}
$$

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

• Eq. [2.65](#page-18-2) becomes an activating Hill function for high bias and sufficient input. Writing Eq. [2.65](#page-18-2) in terms of  $K_T$  and the bias  $c = K_T/K_R$ 

$$
f_{\text{on}} = \frac{(1 + K_T[X])^n}{(1 + K_T[X])^n + L(1 + K_T[X]/c)^n}
$$
\n(2.72)

then if we have high bias,  $c \gg K_T[X]$ ,

$$
f_{\text{on}} \simeq \frac{(1 + K_T[X])^n}{(1 + K_T[X])^n + L}.
$$
\n(2.73)

If too we have sufficient input,  $K_T[X] \gg 1$ , then

$$
f_{\text{on}} \simeq \frac{(K_T[X])^n}{(K_T[X])^n + L}
$$
  
= 
$$
\frac{[X]^n}{\frac{L}{K_T^n} + [X]^n}
$$
 (2.74)

which is a Hill equation (Eq. [2.49\)](#page-11-4) with a Hill number equal to n, the number of regulatory binding sites on each allosteric molecule. These limits reduce the system to having essentially two states:  $T_n \simeq (K_T X)^n T$  and R, with  $[R] = L[T]$ .

• Eq. [2.65](#page-18-2) becomes an inhibiting Hill function for low bias and sufficient input. Writing Eq. [2.65](#page-18-2) in terms of  $K_R$  and the bias  $c = K_T / K_R$ <br> $(1 + cK_R)$ 

$$
f_{\text{on}} = \frac{(1 + cK_R[X])^n}{(1 + cK_R[X])^n + L(1 + K_R[X])^n}
$$
\n(2.75)

then if we have low bias,  $c \ll \frac{1}{K_R[X]},$  $f_{\rm c}$ <br>s, c<br> $t$  in  $\frac{1}{2}$ h<sub>ave</sub>

$$
\frac{(1 + cK_R[X])^n}{(1 + cK_R[X])^n + L(1 + K_R[X])^n}
$$
\n
$$
f_{\text{on}} \simeq \frac{1}{1 + L(1 + K_R[X])^n}.
$$
\n
$$
K_R[X] \gg 1, \text{ then}
$$
\n
$$
f \simeq \frac{1}{1 + L(1 + K_R[X])^n}.
$$
\n(2.76)

If too we have sufficient input,  $K_R[X] \gg 1$ , then put,  $K_R[X] \gg 1$ , then

$$
f_{\text{on}} \simeq \frac{1}{1 + L(K_R[X])^n}
$$
  
= 
$$
\frac{\frac{1}{LK_R^n}}{\frac{1}{LK_R^n} + [X]^n}
$$
 (2.77)

which is also a Hill function (Eq. [2.50\)](#page-12-0).

Allostery can therefore cause an enzyme to switch sharply between active and inactive states Allostery can therefore cause an enzyme to switch sharply between active and inactive states<br>at a threshold concentration of the regulatory molecule. This cooperative behaviour arises because the first regulatory molecule prefers binding to the enzyme's conformational state that favours binding of more regulatory molecules. The bound enzyme will spend more time in this favours binding of more regulatory molecules. The bound enzyme will spend more time in this conformation making it easier for a second regulatory molecule to bind. It then becomes even easier for a third molecule to bind. easier for a third molecule to bind.  $\overline{a}$  $(2.75)$ <br> $(2.75)$ <br>state arise<br>e than the seven by a reaction by a reaction<br> $\frac{1}{2}$ artive vious<br>are stated and are stated and are stated and are stated and are set to the set of the se  $(X])^n$ <br>  $(X])^n$ <br>  $(X])^n$ <br>  $(X)$  $\overline{C_R[X]}$  .  $\overline{C_R[X]}$  .  $\overline{C_R}[X]$ The rations is a summer discussed in the ration of the rational because the rational because of the rational because of the ration of the ow b<br>ifficien.<br>iill function.<br>if easing the association.<br>if  $\operatorname{sig}$ ave so a<br>ther once<br>regu<br>hing mol<br>llin<br>llost the<br>
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O t if the contract of the contr too v<br>ich is<br>tery<br>the bind<br>ation<br>r a t  $f_{\text{or}}$ <br>s,  $c \ll$ <br>tion use a form a receptor-receptor-receptor-receptor-receptor-receptor dimersion and some  $f$ twee<br>is co<br>me who this system are at this system are at  $\frac{1}{2}$ reaction. Although the association. Although the association of the as e and ve k<br>the dissociation reaction reaction processes at the rate of  $\mathbf{g}$ . I.  $(2.3)$ <br>  $(2.3)$ 

# 2.10 Modelling signal transduction III<br>
we are centered a dimer of reaction of reaction in the reaction of the

<span id="page-20-0"></span>We can use an allosteric model to describe activation of the receptors in Fig. [1.](#page-2-1) Consider  $\mathbb{R}$  $\frac{1}{2}$  is the macroscopic rate  $\frac{1}{2}$  is the matrix of the matrix  $\frac{1}{2}$  $\mathbf{r}$ 



tivat  $\mathfrak{g}\in\mathfrak{g}$  $\frac{1}{2}$ en t then the fraction of activated receptors is

= 2f[R] <sup>2</sup> + 2b[R2] (8) are M ↵usion-limited. The units of the macroscopic rate dt f <sup>∗</sup> = 1 + K<sup>∗</sup> [S] 1 + K<sup>∗</sup> [S] + <sup>L</sup>(1 + <sup>K</sup>[S]) (2.78)

from Eq. [2.60.](#page-15-1) The concentration of active receptors is  $[R^*] = f^*R_0$ , and so Eq. [2.58](#page-14-3) becomes

<span id="page-21-1"></span>
$$
\frac{d[A^*]}{dt} \simeq \frac{kR_0(1+K^*[S])}{1+K^*[S]+L(1+K[S])}(A_0-[A^*]).
$$
\n(2.79)

When  $[S] = 0$ , Eq. [2.79](#page-21-1) simplifies

$$
\frac{d[A^*]}{dt} \simeq \frac{kR_0}{1+L}(A_0 - [A^*])
$$
\n(2.80)

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 almost all the active receptors are in the  $R_1^*$  state and not in the  $R^*$ state, then Eq. [2.79](#page-21-1) becomes

$$
\frac{d[A^*]}{dt} \simeq \frac{kR_0 K^*[S]}{L + (K^* + KL)[S]} (A_0 - [A^*])
$$
\n(2.81)

and we recover Eq. [2.40.](#page-9-1)

#### <span id="page-21-0"></span>2.11 Enzyme kinetics

Almost all studies of enzymes start with the framework introduced by Michaelis and Menten, which although approximate is both simple and practical. An enzymatic reaction occurs 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:

$$
\mathbf{E} + \mathbf{S} \xrightarrow[\mathbf{b}]{f} \mathbf{C} \xrightarrow[\mathbf{b}]{k} \mathbf{P} + \mathbf{E}
$$

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

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

<span id="page-21-2"></span>
$$
\frac{d[E]}{dt} = -f[E][S] + (b+k)[C] \n\frac{d[S]}{dt} = -f[E][S] + b[C] \n\frac{d[C]}{dt} = f[E][S] - (b+k)[C] \n\frac{d[P]}{dt} = k[C].
$$
\n(2.82)

Catalysis does not consume the enzyme, and we see that

$$
\frac{d[E]}{dt} + \frac{d[C]}{dt} = 0\tag{2.83}
$$

so that

<span id="page-21-3"></span>
$$
[E] + [C] = [E]_0 + [C]_0 = E_{\text{tot}} \tag{2.84}
$$

where the right-hand side is the total amount of enzyme initially present  $-E_{\text{tot}}$ . Similarly, the substrate is only 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, often true initially, so that almost all the enzyme is bound in a complex with the substrate. The concentration of the complex then does not change with time, although  $|S|$  and [P] do. The concentration of complex remains approximately constant while levels of S remain sufficiently high. We say that [C] is at quasi-steady state because  $d|C|/dt \approx 0$ , but the system as a whole is not at steady state, with  $d[S]/dt < 0$  and  $d[P]/dt > 0$ .

If  $d[C]/dt \simeq 0$ , then

<span id="page-22-1"></span>
$$
f[E][S] = (b+k)[C]
$$
\n(2.85)

from Eqs [2.82.](#page-21-2) Combining Eq. [2.85](#page-22-1) with Eq. [2.84,](#page-21-3) we can show that

$$
[C] \simeq \frac{E_{\text{tot}}[S]}{\frac{b+k}{f} + [S]}
$$
\n(2.86)

and so

<span id="page-22-4"></span>
$$
\frac{d[P]}{dt} \simeq \frac{kE_{\text{tot}}[S]}{\frac{b+k}{f} + [S]}
$$
\n(2.87)

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

Defining

$$
V_{\text{max}} = kE_{\text{tot}} \quad ; \quad K_m = \frac{b+k}{f} \tag{2.88}
$$

we have the Michaelis-Menten equation:

<span id="page-22-0"></span>
$$
\frac{d[P]}{dt} \simeq \frac{V_{\text{max}}[S]}{K_m + [S]}
$$
\n(2.89)

for the initial rate of an enzymatic reaction. The maximum rate of the reaction is given by  $V_{\text{max}}$ 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-Menten constant,  $K_m$ .

We have too that

<span id="page-22-2"></span>
$$
\frac{d[S]}{dt} + \frac{d[C]}{dt} + \frac{d[P]}{dt} = 0
$$
\n(2.90)

because the substrate is either free or in a complex with the enzyme or has been converted into the product. The quasi-steady-state assumption,  $d[C]/dt \simeq 0$ , implies that Eq. [2.90](#page-22-2) becomes

$$
\frac{d[S]}{dt} \simeq -\frac{d[P]}{dt} \tag{2.91}
$$

or

$$
\frac{d[S]}{dt} \simeq -\frac{V_{\text{max}}[S]}{K_m + [S]}
$$
\n(2.92)

from Eq. [2.89,](#page-22-0) another form of the Michaelis-Menten equation.

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

<span id="page-22-3"></span>
$$
\frac{E_{\text{tot}}}{[S]_0 + K_m} \ll 1\tag{2.93}
$$

is necessary for Eq. [2.89](#page-22-0) to hold  $[8]$ , where  $[S]_0$  is the initial concentration of substrate. Eq. [2.93](#page-22-3) implies first that the time taken for the enzyme to bind substrate to build the complex  $C$  is faster than the time taken for the levels of substrate to change, and, second, that a negligible amount of substrate is lost while the complex forms [\[8\]](#page-63-7). The initial condition that we can use with the quasi-steady state approximation is then still  $[S(t=0)] = [S]_0$ , the true initial condition of the system.

In the cell, however, enzymes, such as those in metabolism, often operate in the presence of their product, which competes with the substrate to bind to the enzyme and is inhibiting. The Michaelis-Menten equation then no longer holds. Furthermore, metabolic enzymes usually have more than one substrate [\[9\]](#page-63-8).

#### <span id="page-23-0"></span>2.12 Modelling signal transduction IV

Given our allosteric model of the activation of the receptors in Fig. [1](#page-2-1) (Eq. [2.79\)](#page-21-1), we can consider how the signal propagates within the cell and model the dynamics of kinase  $B$ , which is activated by A<sup>∗</sup> . We will assume that this activation obeys Michaelis-Menten kinetics:

$$
A^* + B \xrightarrow[t_{\overline{b_B}} C_{AB} \xrightarrow{k_B} B^* + A^*
$$

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

$$
\frac{k_B[A^*][B]}{\frac{b_B+k_B}{f_B}+[B]}
$$
\n
$$
(2.94)
$$

from Eq. [2.87.](#page-22-4) Note that it is only active A that catalyses the activation of B, and so  $[A^*]$  is equivalent to  $E_{\text{tot}}$  in Eq. [2.87.](#page-22-4)

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

$$
P + B^* \xrightarrow[\overline{b'_B}]{f'_B} C_{PB} \xrightarrow[k'_B]{} B + P
$$

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

$$
-\frac{k'_{B}[P][B^*]}{\frac{b'_{B}+k'_{B}}{f'_{B}}+[B^*]}.
$$
\n(2.95)

Hence

$$
\frac{d[B^*]}{dt} \simeq \frac{k_B[A^*][B]}{\frac{b_B+k_B}{f_B} + [B]} - \frac{k'_B[P][B^*]}{\frac{b'_B+k'_B}{f'_B} + [B^*]} \tag{2.96}
$$

or

<span id="page-23-1"></span>
$$
\frac{d[B^*]}{dt} \simeq \frac{k_B[A^*](B_0 - [B^*])}{\frac{b_B + k_B}{f_B} + B_0 - [B^*]} - \frac{k'_B[P][B^*]}{\frac{b'_B + k'_B}{f'_B} + [B^*]}
$$
\n(2.97)

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  $[B^*] \ll \frac{b_B^2 + k_B^2}{f_B^2}$ . Eq. [2.97](#page-23-1) then simplifies

$$
\frac{d[B^*]}{dt} \simeq \frac{k_B[A^*](B_0 - [B^*])}{\frac{b_B + k_B}{f_B} + B_0 - [B^*]} - d_B[B^*]
$$
\n(2.98)

where

$$
d_B = \frac{f'_B k'_B [P]}{b'_B + k'_B}.
$$
\n(2.99)

Including similar activation of molecule  $C$  by  $B^*$ , our final model of the cytoplasmic reactions of Fig. [1](#page-2-1) is

<span id="page-24-3"></span>
$$
\frac{d[A^*]}{dt} = \frac{k_A R_0 (1 + K^*[S])}{1 + K^*[S] + L(1 + K[S])} (A_0 - [A^*]) - d_A [A^*]
$$
\n
$$
\frac{d[B^*]}{dt} = \frac{k_B [A^*](B_0 - [B^*])}{\frac{b_B + k_B}{f_B} + B_0 - [B^*]} - d_B [B^*]
$$
\n
$$
\frac{d[C^*]}{dt} = \frac{k_C [B^*](C_0 - [C^*])}{\frac{b_C + k_C}{f_C} + C_0 - [C^*]} - d_C [C^*]
$$
\n(2.100)

assuming that deactivating enzymes, the phosphatase, are present and far from saturation.

#### <span id="page-24-0"></span>2.13 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 the oocyte maturing.

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<sup>∗</sup> ] then

<span id="page-24-1"></span>
$$
[B^*] = [B^*]_{\text{max}} \cdot \frac{[A^*]^{n_B}}{K_B^{n_B} + [A^*]^{n_B}}
$$
\n(2.101)

where  $n_B$  is the Hill number and  $K_B$  is the EC<sub>50</sub> of the activation of B by A<sup>∗</sup>. Similarly, if steady-state  $[C^*]$  is a sigmoidal function of  $[B^*]$  then

<span id="page-24-2"></span>
$$
[C^*] = [C^*]_{\text{max}} \cdot \frac{[B^*]^{n_C}}{K_C^{n_C} + [B^*]^{n_C}}
$$
\n(2.102)

where  $n_C$  is the Hill number and  $K_C$  is the  $EC_{50}$  of  $B^*$ . Inserting Eq. [2.101](#page-24-1) into Eq. [2.102](#page-24-2) gives

$$
[C^*] = [C^*]_{\text{max}} \cdot \frac{\left( [B^*]_{\text{max}} \frac{[A^*]^n B}{K_B^{n_B} + [A^*]^n B} \right)^{n_C}}{K_C^{n_C} + \left( [B^*]_{\text{max}} \frac{[A^*]^n B}{K_B^{n_B} + [A^*]^n B} \right)^{n_C}}.
$$
\n(2.103)

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

$$
[C^*] \simeq [C^*]_{\text{max}} \cdot \frac{[A^*]^{n_{B}n_{C}}}{\frac{K_B^{n_{B}n_{C}} K_C^{n_{C}}}{[B^*]_{\text{max}}^{n_{C}}} + [A^*]^{n_{B}n_{C}}}
$$
(2.104)

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.

<span id="page-25-1"></span>

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.

For example, if each element of the cascade has a Hill number of two 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 one regardless of the number of stages in the cascade (Fig. [3\)](#page-25-1).

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 to become active then it is difficult to generate ultrasensitivity without having to impose restrictions on the concentrations of the enzymes [\[10\]](#page-63-9). 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, then activation of the downstream kinase 'sees' the concentration of the upstream kinase twice, once for each phosphorylation. For a processive kinase, which binds and phosphorylates the substrate twice before dissociating, the concentration of the upstream kinase is seen only once. We therefore might expect activation of a kinase by a distributive upstream kinase to be a sigmoidal function of that upstream kinase's concentration. Where tested, this expectation has been borne out [\[11\]](#page-63-10).

#### <span id="page-25-0"></span>2.14 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 [\[10\]](#page-63-9). 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. [2.89\)](#page-22-0). Zero-order ultrasensitive responses can switch sharply and can have Hill numbers greater than 10.

#### <span id="page-26-0"></span>2.15 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, 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 we can derive these approximations 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.

# <span id="page-27-0"></span>3 Modelling gene expression

Gene expression is fundamental to much of biology and modelling gene expression is fundamental to much of systems biology. We can use equations of chemical reactions to describe binding of proteins to promoters and to describe transcription and translation. To understand the average behaviour of a system, we typically model how occupied the promoters of interest are by transcription factors and RNA polymerase on average.

To proceed, we assume that binding of proteins to the DNA occurs 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 by assuming that the DNA-binding reactions are at equilibrium, but identical expressions can be found too using ideas from statistical mechanics [\[12\]](#page-63-11).

#### <span id="page-27-1"></span>3.1 Modelling constitutive expression

A constitutively expressed gene is unregulated and synthesises mRNA at a constant average rate. 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

$$
P_0 + Q \Longleftarrow P_0^Q
$$

describes the binding of RNA polymerase to the promoter.  $P_0^Q$  $\int_0^Q$  is the complex of the promoter bound by RNA polymerase:



At equilibrium,

<span id="page-27-2"></span>
$$
P_0^Q = K_Q Q P_0 \tag{3.1}
$$

where  $Q$  here represents the number of molecules of  $Q$ , which we can convert into a concentration We expect  $K_Q > 1$  because it is determined by polymerases's binding energy to the DNA,  $\Delta G_b$ ,  $[Q]$  by dividing both sides of the equation by the volume of the cell.  $K_Q$  is an association constant. via  $K_Q = e^{-\frac{\Delta G_b}{RT}}$  [\[12\]](#page-63-11), and  $\Delta G_b < 0$ .

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

$$
P_0 + P_0^Q = n.\t\t(3.2)
$$

Using Eq. [3.1,](#page-27-2) this conservation implies that

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

and so that

<span id="page-27-3"></span>
$$
P_0 = \frac{n}{1 + K_Q Q} \tag{3.4}
$$

which is the average number of promoters that are free and not bound by RNA polymerase. From Eq. [3.1,](#page-27-2) the average number of bound promoters is

$$
P_0^Q = \frac{nK_QQ}{1 + K_QQ}.\tag{3.5}
$$

#### <span id="page-28-0"></span>3.1.1 Modelling transcription

Transcription occurs only when RNA polymerase Q is bound

$$
P_0^Q \xrightarrow{u} P_0^Q + M
$$

where  $u$  is the rate at which RNA polymerase initiates transcription. We assume that the binding of RNAP at the promoter is fast compared to  $u$  and remains at equilibrium so that a bound polymerase replaces the one that leaves the DNA after it finishes transcribing. If the mRNA is degraded

$$
M \xrightarrow{d_M} \varnothing
$$

then the rate equation for the mRNA  $M$  is

<span id="page-28-2"></span>
$$
\frac{dM}{dt} = uP_0^Q - d_M M\tag{3.6}
$$

and the half-life of mRNA is  $\log(2)/d_M$  (see Sec. [2.1.2\)](#page-5-0).

Eq. [3.1](#page-27-2) and Eq. [3.4](#page-27-3) imply that Eq. [3.6](#page-28-2) can be written as

<span id="page-28-4"></span>
$$
\frac{dM}{dt} = \frac{n u K_Q Q}{1 + K_Q Q} - d_M M. \tag{3.7}
$$

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

#### <span id="page-28-1"></span>3.1.2 Modelling translation

Translation is usually modelled as a first-order process with rate, say, v:

$$
M \xrightarrow{v} M + P
$$

for mRNA, M, and protein, P. With first-order degradation of proteins,

$$
P \xrightarrow{d_P} \varnothing
$$

the equation for protein dynamics is then

<span id="page-28-3"></span>
$$
\frac{dP}{dt} = vM - d_P P \tag{3.8}
$$

with  $d_P$  being the protein degradation rate. The half-life of protein is  $\log(2)/d_P$ . In Eq. [3.8,](#page-28-3) M is a function of time and obeys Eq. [3.7.](#page-28-4)

Eq. [3.7](#page-28-4) and Eq. [3.8](#page-28-3) together model constitutive gene expression.

#### <span id="page-29-0"></span>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

$$
\mathbf{P}_0 + \mathbf{R} \Longleftrightarrow \mathbf{P}_1
$$

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

$$
P_0 + Q \Longleftarrow P_0^Q
$$

and initiate transcription with a rate u.

If both these binding reactions are at equilibrium then

<span id="page-29-1"></span>
$$
P_1 = K_R R P_0 \quad ; \quad P_0^Q = K_Q Q P_0 \tag{3.9}
$$

where  $K_R$  and  $K_Q$  are 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 in total, we can write:

$$
P_0 + P_0^Q + P_1 = n \tag{3.10}
$$

Using Eq. [3.9,](#page-29-1) this conservation implies that

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

and so that

$$
P_0 = \frac{n}{1 + K_Q Q + K_R R} \tag{3.12}
$$

which is the average number of promoters that are free and bound by neither the repressor nor RNA polymerase. From Eq. [3.9,](#page-29-1) the average number of promoters that are able to transcribe – have a bound RNA polymerase – is

<span id="page-29-2"></span>
$$
P_0^Q = \frac{nK_QQ}{1 + K_QQ + K_RR}.\tag{3.13}
$$

The rate equation describing transcription is then

$$
\frac{dM}{dt} = uP_0^Q - d_M M\tag{3.14}
$$

or, from Eq. [3.13,](#page-29-2)

<span id="page-30-1"></span>
$$
\frac{dM}{dt} = \frac{n u K_Q Q}{1 + K_Q Q + K_R R} - d_M M. \tag{3.15}
$$

We may write Eq. [3.15](#page-30-1) as

$$
\frac{dM}{dt} = \frac{\left(\frac{n u K_Q Q}{1 + K_Q Q}\right)}{1 + \left(\frac{K_R}{1 + K_Q Q}\right) R} - d_M M\tag{3.16}
$$

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 further write

<span id="page-30-2"></span>
$$
\frac{dM}{dt} = u_{\text{max}} \left[ \frac{1}{1 + \frac{R}{K_1}} \right] - d_M M \tag{3.17}
$$

where the maximum rate of transcription is  $u_{\text{max}} = \frac{n u K_Q Q}{1 + K_Q Q}$  $\frac{n\mu_{\mathbf{N}_Q Q}}{1+K_Q Q}$  and the half-maximal number of repressors is  $K_1 = \frac{1+K_QQ}{K_P}$  $\frac{K_{QQ}}{K_{R}}$ . Note that both these quantities are functions of the numbers of free RNA polymerase, Q.

We again model translation as a first-order process:

<span id="page-30-4"></span>
$$
\frac{dP}{dt} = vM - d_P P \tag{3.18}
$$

where  $M$  satisfies Eq. [3.17.](#page-30-2)

#### <span id="page-30-0"></span>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 will proceed as before and consider the binding of activator, A, to the free promoter

$$
\mathbf{P_0} + \mathbf{A} \xleftarrow[]{} \mathbf{P_1}
$$

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

$$
P_1 + Q \Longleftarrow P_1^Q
$$

We will assume further that transcription only occurs from this  $P_1^Q$  $_1^{\mathcal{Q}}$  state.



When the number of promoters is conserved and all reactions involving DNA binding are at equilibrium

<span id="page-30-3"></span>
$$
P_1 = K_A A P_0 \quad ; \quad P_1^Q = K'_Q Q P_1,\tag{3.19}
$$

where  $K_A$  and  $K_Q'$  are association constants, and

<span id="page-31-1"></span>
$$
P_0 + P_1 + P_1^Q = n \tag{3.20}
$$

where  $n$  is the number of promoters.

Combining Eq. [3.19](#page-30-3) and Eq. [3.20](#page-31-1) implies that

$$
P_1^Q = \frac{nK'_Q K_A A Q}{1 + K_A A + K'_Q K_A A Q} \tag{3.21}
$$

for the average number of promoters occupied by RNA polymerase. If  $u$  is the rate of transcription when both polymerase and activator are bound then mRNAs obey

$$
\frac{dM}{dt} = \frac{uK'_Q QK_A A}{1 + K_A A + K_A K'_Q A Q} n - d_M M
$$
\n(3.22)

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

<span id="page-31-4"></span>
$$
\frac{dM}{dt} = \frac{(nuK'_{Q}Q)K_{A}A}{1 + (1 + K'_{Q}Q)K_{A}A} - d_{M}M
$$
\n
$$
= \frac{\left(\frac{nuK'_{Q}}{1 + K'_{Q}Q}\right)(1 + K'_{Q}Q)K_{A}A}{1 + (1 + K'_{Q}Q)K_{A}A} - d_{M}M
$$
\n
$$
= u_{\max} \left[\frac{\frac{A}{K_{1}}}{1 + \frac{A}{K_{1}}}\right] - d_{M}M
$$
\n(3.23)

with  $u_{\text{max}} = \frac{n u K_Q' Q}{1 + K_Q' Q}$  $\frac{n u K_Q Q}{1 + K_Q' Q}$  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.

#### <span id="page-31-0"></span>3.4 Activation by two activators

We can extend this approach 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 activators bind both sites. Denoting  $P_{00}$  as the free promoter,  $P_{10}$  and  $P_{01}$  as the promoter when a transcription factor binds one site, and  $P_{11}$  as the promoter when transcription factors bind both sites, then we have

$$
P_{00} + A \xrightarrow{\longrightarrow} P_{10} \quad \text{and} \quad P_{00} + A \xrightarrow{\longrightarrow} P_{01}
$$

and

$$
P_{01} + A \xrightarrow{\longrightarrow} P_{11} \quad \text{and} \quad P_{10} + A \xrightarrow{\longrightarrow} P_{11}
$$

If these reactions are at equilibrium, we can write

<span id="page-31-2"></span>
$$
P_{10} = K_{10} A P_{00} \quad ; \quad P_{01} = K_{01} A P_{00} \tag{3.24}
$$

and

<span id="page-31-3"></span>
$$
P_{11} = \tilde{K}_{10} A P_{01} \quad ; \quad P_{11} = \tilde{K}_{01} A P_{10} \tag{3.25}
$$



with  $K_{10}$ ,  $K_{01}$ ,  $\tilde{K}_{10}$ , and  $\tilde{K}_{01}$  being association constants and the tilde denoting binding when another activator is already bound.

Eq. [3.24](#page-31-2) and Eq. [3.25](#page-31-3) form a thermodynamic cycle and so a relationship exists between the equilibrium association constants

<span id="page-32-0"></span>
$$
K_{01}\tilde{K}_{10} = K_{10}\tilde{K}_{01} \tag{3.26}
$$

because at equilibrium there should be nothing unique about the route taken to form  $P_{11}$ , whether the activator binds initially to either the first or the second binding site. Finally, let us assume that RNA polymerase can only bind to the promoter when activators bind both their sites  $P_{11} + Q \rightleftharpoons P_{11}^Q$ 

and so

$$
P_{11}^Q = K'_Q Q P_{11} \tag{3.27}
$$

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

Again we have a fixed number of promoters

$$
P_{00} + P_{10} + P_{01} + P_{11} + P_{11}^Q = n \tag{3.28}
$$

which implies that

$$
P_{00} + K_{10}AP_{00} + K_{01}AP_{00} + \tilde{K}_{10}K_{01}A^2P_{00} + \tilde{K}_{10}K_{01}K'_QQA^2P_{00} = n \tag{3.29}
$$

11

and so

$$
P_{11}^{Q} = \frac{nK'_{Q}\tilde{K}_{10}K_{01}QA^{2}}{1 + K_{10}A + K_{01}A + \tilde{K}_{10}K_{01}A^{2} + \tilde{K}_{10}K_{01}K'_{Q}QA^{2}}
$$
(3.30)

is the average number of promoters occupied by RNA polymerase.

Letting

$$
\tilde{K}_{10} = K_i K_{10} \tag{3.31}
$$

with  $K_i$  greater than one and determined by the free energy of interaction between both activators when bound at the promoter,  $K_i = e^{-\frac{\Delta G_{\text{int}}}{RT}}$ , then the number of mRNAs obeys

<span id="page-32-1"></span>
$$
\frac{dM}{dt} = \frac{u n K_Q' Q K_i K_{10} K_{01} A^2}{1 + K_{10} A + K_{01} A + K_i K_{10} K_{01} A^2 + K_i K_{10} K_{01} K_Q' Q A^2} - d_M M \tag{3.32}
$$

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

$$
\frac{dM}{dt} = u_{\text{max}} \left[ \frac{\frac{A^2}{K_2^2}}{1 + \frac{A}{K_1} + \frac{A^2}{K_2^2}} \right] - d_M M \tag{3.33}
$$

with  $u_{\text{max}} = \frac{u n K_Q' Q}{1 + K_Q' Q}$  $\frac{u n K_Q Q}{1 + K_Q Q}$ ,  $K_1^{-1} = K_{01} + K_{10}$ , and  $K_2^{-2} = K_i K_{10} K_{01} (1 + K_Q Q)$ . The maximal Hill number is two.

Note that if  $\tilde{K}_{10} = K_i K_{10}$  then  $\tilde{K}_{01} = K_i K_{01}$  because the energy of interaction between the activators is the same in both cases. Note too that Eq. [3.26](#page-32-0) is then satisfied, as expected.

#### <span id="page-33-0"></span>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:

$$
P_0 + Q \xrightleftharpoons P_Q
$$

with  $P_Q = K_Q Q P_0$ . If  $u_{\ell}$  is the rate of transcription from this state — such unregulated transcription is sometimes called leakage, then Eq. [3.32](#page-32-1) becomes

$$
\frac{dM}{dt} = n \frac{u_{\ell} K_{Q} Q + u_{Q} K_{i} K_{10} K_{01} A^{2}}{1 + K_{Q} Q + K_{10} A + K_{01} A + K_{i} K_{10} K_{01} A^{2} + K_{i} K_{10} K_{01} K_{Q}^{\prime} Q A^{2}} - d_{M} M \tag{3.34}
$$

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

<span id="page-33-2"></span>
$$
\frac{dM}{dt} = \frac{u_{\text{basal}} + u_{\text{max}} \times \frac{A^2}{K_2^2}}{1 + \frac{A}{K_1} + \frac{A^2}{K_2^2}} - d_M M \tag{3.35}
$$

but now with  $K_1^{-1} = \frac{K_{01} + K_{10}}{1 + K_O Q}$  $\frac{K_{01}+K_{10}}{1+K_QQ}$  and  $K_2^{-2} = K_i K_{10} K_{01} \frac{1+K'_QQ}{1+K_QQ}$  $\frac{1+\kappa_Q\sigma}{1+\kappa_QQ}$  and with a basal rate of transcription of  $u_{\text{basal}} = \frac{u_{\ell} n K_Q Q}{1 + K_Q Q}$  $\frac{u_{\ell} n K_Q Q}{1 + K_Q Q}$ . As before,  $u_{\text{max}} = \frac{u n K_Q Q}{1 + K_Q Q}$  $\frac{\dim Q}{1+K'_{Q}Q}$ . For the activators to be efficient, RNAP should prefer to bind to the promoter when two activators have already bound,  $K'_{Q} > K_{Q}$ , and the rate of transcription should be highest from this state, so that  $u > u_{\ell}$ .

#### <span id="page-33-1"></span>3.5 General regulation

There is a pattern in the expressions for the average rate of transcription, one expected from statistical mechanics [\[12\]](#page-63-11). Each term in the denominator represents a possible state of the promoter: we represent the free state by the number 1 and a bound state by the product of the association constants for each binding event times the number of ways those binding events can occur. Each term in the numerator represents a state of the promoter from which transcription can occur. We multiply each of these terms by their rate of transcription and 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. The promoter then has



black and the state of the five states: free, both by a repressor, and l transcription is reaction proceeds at the rate f Reaction process at the rate f Reaction process are the rate f Reaction process at the rate f Reaction process and the rate for  $\mathbf{r}_1$  and  $\mathbf{r}_2$  are the rate for  $\mathbf{r}_2$  and  $\mathbf{r}_$ transcription is five states: 1<br>both reactions. The dimersions. The dimersions. The dimersions. The dimersions. The dimersions 2011<br>Sections. The dimersions. The dimersions. The dimersions of the dimersions. The dimersions of the dimersion 1.1.1 Example: dimerization the states. Het, bound by polymerase, one site bound by a repressor, the other site bound<br>by a repressor, and both sites bound by a repressor. The denominator of the average rate of<br>transcription is e o<br>the  $\vdots$  then  $\vdots$  of  $\vdots$  $\frac{1}{2}$  $\sum_{i=1}^{n}$ reactions in the reaction of reactions in the reaction of  $\frac{1}{\sqrt{2\pi}}$ five the matrix of the matrix of the matrix of the matrix of the matrix  $\mathbf{r}$ a n<br>nsc<br>th R molecules are involved in both reactions. The dimer, because on<br>Because of dimer forms or disponents or dimer forms or disponents. Summing Eq. 8 and twice Eq. 8 and twice Eq. 9 and twice Eq. 8 and twice Eq. 8 and twice Eq. 8 and twice Eq. 9 and twice Eq. 9 and twice Eq. 9 an rased that  $\frac{1}{q}$  $\begin{aligned} \mathsf{P} \mathsf{R} \mathsf{P} \mathsf{R} \mathsf{R$ st.<br>a reactions.<br>th e b<br>
essc $+$  *F*<br>  $K_Q$ <br>  $K_{11}$  res st:<br>a r<br>th<br>ree<br>pro five states: free, bound by polymerase, one site bound by a repressor, the other site bound

$$
1 + K_Q Q + K_{01} R + K_{10} R + K_{11} R^2 \tag{3.36}
$$

and the macroscope rate  $\mathfrak{m}$  is  $\mathfrak{m}$  and  $\mathfrak{m}$ and the numerator is  $\mathbf{c}$  on differentiates. Summing  $\mathbf{c}$ and the numerator is

$$
1 + K_Q Q + K_{01} R + K_{10} R + K_{11} R^2
$$
 (3.36)  
merator is  

$$
nu \times K_Q Q.
$$
 (3.37)  
or determine the association operator  $K$ , for two processes binding simultaneously to

five states: fr<br>by a repressor<br>transcription i<br>and the numer<br>Three factors of<br>the promoter:<br>constant; the energy of inte<br>Therefore the<br>which we can<br>The maximal  $\begin{array}{c} \epsilon \\ R \\ \epsilon \\ \epsilon \\ \epsilon \\ \eta \end{array}$ constant; the change in free energy of another binding, or Three factors determine the association constant  $K_{11}$  for two<br>the promoter: the change in free energy of one repressor bine Therefore the number of mRNAs satisfies the rate equation<br>  $\frac{dM}{dx} = mc^2$ e of<br>the g sinnes<br> $g$  sinnes<br> $K_1$ in the molecule of model  $\frac{1}{2}$  and  $\frac{1}{2}$ n d<br>tio<br>ve  $\mathbf{r}$  : essor bii: rgy of another binding, or its association<br>
wo bound repressors. As before, we<br>
s satisfies the rate equation:<br>  $K_Q Q$ A Rthe promoter: the change in free energy of one repressor binding, which determines its association Three factors determine the association constant  $K_{11}$  for two repressors binding simultaneously to the promoter: the change in free energy of one repressor binding, which determines its association  $\begin{aligned} \text{sym} & 4 \text{ is } t \text{$ po<br>tes<br>1<br>asset<br>free<br>en<br>RN fried by the unit of the contract of  $\Gamma$ ee<br>pr<br>sta<br>rgy<br>ere because on<br>isociates. Summing the disconnel<br>or disconnel or disconnel twice Eq. 8 and twic imply<br>implying the that<br>interface that  $e$  of the g sin in terms of  $K_1$ in the model of model is the model of  $d_M$ energy of interaction between two bound repressors. As before, we have  $K_{11} = K_{01}K_{10}K_i$ . constant; the change in free energy of another binding, or its association constant; and the free  $\begin{align} \text{sum} \ \text{ciat} \ \text{energy} \ \text{two} \ \text{s} \ \text{s} \end{align}$ reactions rates, for reactions, and the concentrations, and interactions, and it is also that the concentration  $\frac{1}{1+\frac{1}{2}}$ five divided in the manner of the material control of the material of the mate because<br>rgy<br>ere  $\begin{array}{c} \text{sum} \ \text{block} \ \text{sum} \ \text{$  $\int_{\text{C}}^{\infty}$ <br>tisf<br> $\frac{1}{2}$ 

Therefore the number of mRNAs satisfies the rate equation:  
\n
$$
\frac{dM}{dt} = nu \frac{K_Q Q}{1 + K_Q Q + K_{01} R + K_{10} R + K_i K_{10} K_{01} R^2} - d_M M
$$
\n(3.38)

which which we can write as

<span id="page-34-1"></span>
$$
\frac{dM}{dt} = \frac{nuK_QQ}{1 + K_QQ} \left[ \frac{1}{1 + \frac{K_{01} + K_{10}}{1 + K_QQ}R + \frac{K_i K_{10} K_{01}}{1 + K_QQ}R^2} \right] - d_M M. \tag{3.39}
$$

<span id="page-34-0"></span>The maximal Hill number is two.

#### $3.6$ 3.6 Including non-specific binding to DNA

 $\operatorname{strong}$  $\lambda$ ltho Although transcriptional regulators have a preferred DNA sequence, one that they bind to strongly, there will be similar sequences in the genome where the regulator may bind weakly. We can include the effect of this non-specific binding in our models of transcriptional regulation [\[12\]](#page-63-11).

> Let's begin with RNA polymerase. As before, we have specific binding to the promoter of interest,  $P_0$ ,

$$
P_0 + Q \Longleftarrow P_0^Q
$$

but no but now we include non-specific binding to other sites on the DNA, which we will call  $N$ :

$$
N + Q \Longleftarrow N^Q
$$

We expect the number of non-specific binding sites,  $n_Q$  say, to be much larger than the number of specific ones:  $n_Q \gg n$ .

With both binding reactions at equilibrium

<span id="page-35-0"></span>
$$
P_0^Q = K_Q Q P_0 \quad ; \quad N^Q = K_Q^N N Q \tag{3.40}
$$

for association constants  $K_Q$  and  $K_Q^N$ . Writing  $m_Q$  for the number of RNA polymerase molecules available to transcribe our gene of interest and providing this number does not change over the time we wish to model, we have that

$$
Q + P_0^Q + N^Q = m_Q \tag{3.41}
$$

or

$$
Q + K_Q Q P_0 + K_Q^N N Q = m_Q. \tag{3.42}
$$

The number of free polymerases, Q, satisfies

<span id="page-35-1"></span>
$$
Q = \frac{m_Q}{1 + K_Q P_0 + K_Q^N N}.\tag{3.43}
$$

The number of polymerases binding non-specifically should be much greater than the number binding specifically:  $N^Q \gg P_0^Q$  $b_0^Q$ , or  $K_Q^N N \gg K_Q P_0$  from Eq. [3.40,](#page-35-0) because there are so many more non-specific binding sites. Therefore

<span id="page-35-2"></span>
$$
Q \simeq \frac{m_Q}{1 + K_Q^N N} \tag{3.44}
$$

from Eq. [3.43.](#page-35-1)

We can simplify further. The number of non-specific binding sites satisfies

$$
N + N_Q = n_Q \tag{3.45}
$$

or

$$
N = \frac{n_Q}{1 + \frac{N_Q}{N}}
$$
\n
$$
\simeq n_Q \tag{3.46}
$$

because we expect most non-specific sites to be free,  $N_Q \ll N$ . Eq. [3.44](#page-35-2) becomes

<span id="page-35-3"></span>
$$
Q \simeq \frac{m_Q}{1 + K_Q^N n_Q}.\tag{3.47}
$$

From Eq. [3.47,](#page-35-3) non-specific binding reduces the number of Q molecules available to bind specifically to the promoter. The more non-specific binding sites, the greater  $n_Q$ , and the stronger the association constant for binding to these sites, the greater  $K_Q^N$ , the fewer polymerases are available to bind specifically. If there is no non-specific binding,  $n_Q = 0$ , and  $Q \simeq m_Q$ .

Although we focused on RNA polymerase, the same argument holds for any transcriptional regulator, and so the number of free repressor molecules is

<span id="page-35-4"></span>
$$
R \simeq \frac{m_R}{1 + K_R^N n_R} \tag{3.48}
$$

if there are  $m_R$  in total and  $K_R^N$  is the association constant for non-specific binding with  $n_R$ non-specific binding sites. For an activator

$$
A \simeq \frac{m_A}{1 + K_A^N n_A} \tag{3.49}
$$

with the parameters defined similarly.

With these expressions, we can add non-specific binding to any of the equations for transcriptional regulation we have already derived. Consider Eq. [3.15](#page-30-1) for regulation by RNA polymerase and a repressor

$$
\frac{dM}{dt} = \frac{n u K_Q Q}{1 + K_Q Q + K_R R} - d_M M. \tag{3.50}
$$

This equation becomes

$$
\frac{dM}{dt} = \frac{n u K_Q \cdot \frac{m_Q}{1 + K_Q^N n_Q}}{1 + K_Q \cdot \frac{m_Q}{1 + K_Q^N n_Q} + K_R \cdot \frac{m_R}{1 + K_R^N n_R}} - d_M M \tag{3.51}
$$

using Eq. [3.47](#page-35-3) and Eq. [3.48.](#page-35-4) Non-specific binding affects transcription by reducing the number of available regulators, from  $m_Q$  for polymerase, decreasing transcription, and from  $m_R$  for the repressor, increasing transcription.

#### <span id="page-36-0"></span>3.7 Modelling signal transduction V

We can now add to the model of Sec. [2.12](#page-23-0) the expression of the reporter gene in Fig. [1,](#page-2-1) which  $C^*$  activates.

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

$$
C^* \xrightarrow[b_n]{f_n} C_n^*
$$

which, if at equilibrium, implies that

$$
[C_n^*] = \frac{f_n}{b_n} [C^*].
$$
\n(3.52)

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  $G, m<sub>G</sub>$ , satisfies, following Eq. [3.23,](#page-31-4)

<span id="page-36-1"></span>
$$
\frac{d[m_G]}{dt} = u_G \frac{\frac{[C_n^*]}{K_{C^*}}}{1 + \frac{[C_n^*]}{K_{C^*}}} - d_m[m_G]
$$
\n(3.53)

with a maximal transcription rate of  $u_G$  and a degradation rate  $d_m$  of the mRNA. Following Eq. [3.18,](#page-30-4) the protein  $G$  obeys

<span id="page-36-2"></span>
$$
\frac{d[G]}{dt} = v[m_G] - d_G[G] \tag{3.54}
$$

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

Eq. [2.100](#page-24-3) with Eq. [3.53](#page-36-1) and Eq. [3.54](#page-36-2) are the complete model of the pathway of Fig. [1,](#page-2-1) from the input  $S$  to the output  $G$ .

# <span id="page-37-0"></span>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 system with positive feedback may have two stable steady states. If the system starts from one set of initial conditions and evolves with time, it will always eventually reach one steady state; if the same system starts from a different set of initial conditions, it will always eventually reach the other steady state. Each steady state has its own basin of attraction defined as all initial concentrations that evolve to that steady state, and each initial condition 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.

#### <span id="page-37-1"></span>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: activating the last kinase of the cascade by adding the hormone progesterone causes new synthesis of the MAP kinase kinase kinase Mos. There is thus positive feedback: more activated Mos causes more activated MAP kinase, which in turn generates more activated Mos by increasing Mos's synthesis.

Following Ferrell *et al.* [\[13\]](#page-63-12), we consider three processes that control levels of Mos. First, there is a basal rate of synthesis that depends on progesterone:

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

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:

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

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

$$
degradation = -[Mos]
$$
 (4.3)

measuring units of time in units of the lifetime of Mos so that the coefficient of [Mos] is one.

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

<span id="page-37-2"></span>
$$
\frac{d[\text{Mos}]}{dt} = k_b[p] + f \frac{[\text{Mos}]^n}{K^n + [\text{Mos}]^n} - [\text{Mos}].\tag{4.4}
$$

Typical parameter values are  $K = 20$  nM,  $n = 5$ ,  $k_b = 0.2$ , and  $f = 40$  [\[13\]](#page-63-12).

At steady state, the rate of synthesis of Mos equals its rate of degradation. Therefore to find steady-state values, we can plot the total synthesis rate and the total degradation rate both as a function of Mos with any intersections between these two curves determining a steady-state concentration of Mos (Fig. [4A](#page-38-0)). Notice that if the rate of synthesis of Mos is not ultrasensitive but hyperbolic, then the system would have only one stable steady state and no switch-like behaviour (Fig. [4B](#page-38-0)).

<span id="page-38-0"></span>

Figure 4: We can find steady-state solutions of Eq. [4.4](#page-37-2) using a graphical construction: the intersection of a curve describing the production rate of [Mos] with a curve describing its degradation rate gives the steady-state [Mos] 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.

<span id="page-38-1"></span>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 initiates 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\)](#page-38-1).



Figure 5: The phase portrait when  $[p] = 20$  nM (see Fig. [6\)](#page-39-1). 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 [\[14\]](#page-63-13). 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: there has been a bifurcation.

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 over time 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.

<span id="page-39-1"></span>

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

As the concentration of pheromone increases from zero, one stable and the unstable steady state approach each other. At the bifurcation point, one 'annihilates' the other, and both disappear (at  $[p] \simeq 47$  nM). The system then has only one steady state. We call this disappearance of a stable and an unstable steady state a saddle-node bifurcation [\[14\]](#page-63-13). Such a bifurcation can also create a stable and an unstable node if, for example, pheromone now decreases.

#### <span id="page-39-0"></span>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 system parameter. For the MAPK system, we can plot the steady-state values of protein as a function of the progesterone concentration  $[p]$  (Fig. [7\)](#page-40-1).

<span id="page-40-1"></span>For low  $[p]$ , there are two stable steady states for Mos (Fig. [6\)](#page-39-1); for high  $[p]$  there is one steady state (Fig. [6\)](#page-39-1). Usually we mark stable steady states on bifurcation diagrams with solid lines and unstable steady states with fainter lines. The signalling system can therefore act as a switch with the steady-state concentration of Mos jumping from a low to a high value as the bifurcation parameter – here  $[p]$  – changes.



Figure 7: A bifurcation diagram showing the steady-state concentrations of Mos as a function of the concentration of pheromone. Stable steady states are in either blue or red; unstable steady states are in black. A saddle-node bifurcation occurs at  $[p] \simeq 47$  nM. The stable steady states of Fig. [6](#page-39-1) are in red.

Bistable systems typically show history-dependent, or hysteretic, behaviour. If we increase [p] 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 its exposure to the high progesterone concentration.

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

#### <span id="page-40-0"></span>4.3 A genetic switch: a two dimensional example of a saddle-node bifurcation

In systems with more than one chemical species, bistability and saddle-node bifurcations occur analogously to the one dimensional case.

For example, consider a protein that activates its own expression [\[14\]](#page-63-13). Writing M for mRNA and P for protein, we can model this two dimensional system as

<span id="page-40-2"></span>
$$
\frac{dM}{dt} = u_b + \frac{u^{pn}}{K^n + P^n} - d_M M \quad ; \quad \frac{dP}{dt} = M - d_P P \tag{4.5}
$$

with a Hill function describing the activation of transcription by  $P$ . Here  $d_M$  is the rate of degradation of mRNA;  $d<sub>P</sub>$  is the rate of degradation of protein; u is the maximal rate of transcription induced by  $P$ ; and  $u<sub>b</sub>$  is a basal rate of transcription. The system has positive feedback because high levels of protein cause higher rates of transcription and so even higher levels of protein.

<span id="page-41-0"></span>

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

In two dimensions, we often use graphical approaches 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 [\[14\]](#page-63-13). These curves are

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

from Eq. [4.5,](#page-40-2) and we plot both curves in the same  $P-M$  plane (Fig. [8\)](#page-41-0). The steady states are the points where the nullclines intercept: at these points,  $dM/dt$  and  $dP/dt$  are both zero.

The genetic switch of Fig. [8](#page-41-0) 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 attracted towards and then repelled from the saddle point. An unstable node in contrast repels all systems that are initialised sufficiently near it.

A phase portrait shows graphically the dynamics of the system. From Eq. [4.5,](#page-40-2)  $dM/dt$  is negative above the  $dM/dt = 0$  nullcline, and the dynamics there decreases M;  $dM/dt$  is positive below the  $dM/dt = 0$  nullcline, and the dynamics there increases M (Fig. [8\)](#page-41-0). Similarly,  $dP/dt$  is positive above the  $dP/dt = 0$  nullcline, and the dynamics increases P;  $dP/dt$  is negative below the  $dP/dt = 0$  nullcline, and the dynamics decreases P. Using arrows to indicate the local direction of the dynamics, we can find the phase portrait (Fig. [9\)](#page-42-0).

<span id="page-42-0"></span>

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 relative to their levels at the unstable steady state.

As we change the degradation rate of protein,  $d<sub>P</sub>$ , the system undergoes two saddle-node bifurcations. The number of intersections of the nullclines changes from one to three to one (Fig. [10\)](#page-43-0). As we change  $d_P$ , a stable steady state, the node, and an unstable steady state, the saddle, either can approach and annihilate each other and thus remove bistability or can be simultaneously created and thus generate bistability. When the rescaled  $d_P \simeq 0.8$ , a saddle and a node appear if  $d<sub>P</sub>$  is increasing and disappear if  $d<sub>P</sub>$  is decreasing. Similarly, when the rescaled  $d_P \simeq 1.3$ , a node and a saddle point disappear if  $d_P$  is increasing and appear if  $d_P$  is decreasing (Fig. [10\)](#page-43-0). Saddle-node bifurcations can create and destroy bistability in all dimensions.

<span id="page-43-0"></span>

Figure 10: When we change  $d_p$ , the system undergoes two saddle-node bifurcations: one at the rescaled  $d_P \simeq 0.8$  and the other at  $d_P \simeq 1.3$  (not shown). Protein and mRNA are relative to their levels at the unstable steady state when  $d_P = 0.002 \text{ s}^{-1}$ , and we measure  $d_P$  relative to  $0.002 \text{ s}^{-1}$ .

The system exhibits hysteresis, or history-dependent behaviour. If we increase  $d_P$  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\)](#page-44-0). If we decrease  $d_P$  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\)](#page-44-0). The value of the threshold when the jump occurs depends on the history of the change in  $d<sub>P</sub>$ .

<span id="page-44-0"></span>

Figure 11: The bifurcation diagram for the genetic switch. The stable steady states are in blue and the unstable steady states in grey. The stable-steady states of Fig. [10](#page-43-0) are in shades of red. Protein and mRNA are relative to their levels at the unstable steady state when  $d_P = 0.002 \text{ s}^{-1}$ , and we measure  $d_P$  relative to 0.002 s<sup>-1</sup>.

This behaviour is general. A system with positive feedback can exhibit hysteresis because the value of the bifurcation parameter at which the system jumps — undergoes a bifurcation is history-dependent: if the system was previously in the high state, then the threshold value at the bifurcation is different from the threshold value if the system was previously in the low state. Observing hysteresis experimentally is usually considered sufficient proof that a system is bistable; observing bimodality, however, is consistent with bistability but insufficient to prove bistability.

## <span id="page-45-0"></span>5 Negative feedback and oscillations

A limit cycle is an isolated and closed trajectory in phase space [\[14\]](#page-63-13). Remember that phase space is the space where we plot the concentration of each chemical species along each axis. An isolated trajectory means that neighbouring trajectories either spiral towards the closed trajectory or spiral away from it. 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 oscillates.

#### <span id="page-45-1"></span>5.1 Degradation stabilises molecular numbers

Degradation stabilises protein numbers. Consider a protein  $P$  with constitutive expression

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

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

$$
k = d_P P^*.
$$
\n<sup>(5.2)</sup>

If levels of P fluctuate higher than  $P^*$ , then the rate of synthesis is unchanged but the rate of degradation increases,  $d_P P > d_P P^* = k$ , 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,  $d_P P \langle d_P P^* = k$ , allowing synthesis to dominate and protein levels to regain steady state.

#### <span id="page-45-2"></span>5.2 Negative feedback is stabilising

Negative feedback acts to reduce perturbations to a system, providing an additional restoring process to degradation.

Consider a gene that is negatively auto-regulated 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 auto-regulation. This negative regulation generates negative feedback in the system. At steady state,  $P = P^*$  and

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

so that the rate of synthesis of P equals its rate of degradation.

If levels of  $P$  fluctuate higher than  $P^*$ , then

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

The auto-negative regulation increases repression, decreasing the synthesis rate, because there are more proteins to bind P's promoter. Levels of P fall towards  $P^*$ . If levels of P fluctuate lower than  $P^*$ , then

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

The repression lessens, increasing the synthesis rate, because there are fewer proteins to bind the promoter. Levels of  $P$  rise towards  $P^*$ .

#### <span id="page-46-0"></span>5.3 Delayed negative feedback can cause oscillations

Delayed negative feedback can be destabilising and generate oscillations providing both the feedback is sufficiently strong and the delay is sufficiently long.

Consider again a negatively auto-regulated gene. At steady state, the synthesis rate exactly matches the degradation rate. If levels of protein fluctuate above average levels, synthesis falls because repression increases. If the fall in synthesis is delayed, however, then levels of protein will rise higher than those for a system without a delay. Once levels of protein do return to the average, there is a mismatch between the current levels of protein and the synthesis rate. This synthesis rate is determined by the higher levels of protein that existed earlier, because of the delay. Synthesis is therefore too low compared to the current rate of degradation, which is determined by the current levels of protein. Protein levels do not stay at the average, but undershoot it. After undershooting, levels of proteins will eventually start to increase and return towards the average, but when they reach the average the synthesis rate will again not match the degradation rate. Now the lower levels of protein that existed earlier determine the synthesis rate, and synthesis is too high compared to degradation. Protein levels overshoot the average, and a cycle initiates. The delays in negative feedback cause protein levels to alternatively undershoot and overshoot their average level; the system oscillates.



rigure 12. Regulated gene if the negative feedback is sufficiently strong and sufficiently delayed. When proteins Figure 12: Negative feedback can cause oscillations in the levels of proteins (blue) of a negatively autoreach their average level (asterisks), the delay causes a mismatch between the synthesis rate and the degradation rate so that the levels of protein continually undershoot and overshoot – they oscillate.

#### <span id="page-46-1"></span>5.4 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 synchronised by environmental signals, such as light and temperature. They are also temperature compensated, persisting over a range of temperatures.

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

In *Drosophila*, the basis of the circadian rhythm is delayed negative feedback through negative auto-regulation 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. A kinase called DBT (double-time) phosphorylates PER in the cytoplasm, and this phosphorylation is necessary for PER's degradation. PER can, however, exist as both a monomer and a dimer. Although DBT phosphorylates both, only the dimer represses the per gene.

<span id="page-47-1"></span>We will follow the model of Tyson *et al.*  $[15]$  (Fig. [13\)](#page-47-1) to explore how the genetic network in Drosophila generates oscillations. Tyson *et al.* assume that the PER dimer rapidly equilibrates between the cytoplasm and the nucleus and that the interconversion of PER monomers and dimers is also at equilibrium.



Figure 13: The model of circadian rhythms of Tyson *et al.* involves a delayed negative feedback where the transcription factor PER represses its own expression when it is a dimer and when it is imported into the nucleus in a complex with TIM. Tyson *et al.* choose not to model TIM explicitly, but include its effects by having different rates of degradation of PER monomers and dimers by DBT.

#### <span id="page-47-0"></span>5.4.1 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 proceed with Michaelis-Menten reactions and, denoting  $P_1$  for PER monomers and  $P_2$  for PER dimers, we have

$$
D + P_1 \xrightarrow[\overline{b_1}]{f_1} C_1 \xrightarrow[k_1]{} P_1^* + D
$$

and

$$
D + P_2 \xrightarrow[t_2]{f_2} C_2 \xrightarrow[k_2]{k_2} P_2^* + D
$$

where we use D to denote the kinase DBT.

As before (see Eq. [2.87\)](#page-22-4), we assume that both  $C_1$  and  $C_2$ , the kinase-substrate complexes, are at quasi-steady state. Then

$$
\frac{dC_1}{dt} = f_1 D P_1 - (b_1 + k_1) C_1 \simeq 0
$$
  
\n
$$
\frac{dC_2}{dt} = f_2 D P_2 - (b_2 + k_2) C_2 \simeq 0
$$
\n(5.7)

and therefore

<span id="page-48-0"></span>
$$
C_1 \simeq \frac{f_1 D P_1}{b_1 + k_1} \quad ; \quad C_2 \simeq \frac{f_2 D P_2}{b_2 + k_2}.
$$
\n
$$
(5.8)
$$

The total amount of kinase,  $D_T$ , is fixed, and  $D + C_1 + C_2 = D_T$ . This conservation law and Eq. [5.8](#page-48-0) implies that

<span id="page-48-1"></span>
$$
D = \frac{D_T}{1 + \frac{f_1 P_1}{b_1 + k_1} + \frac{f_2 P_2}{b_2 + k_2}}.\tag{5.9}
$$

Consequently, the rate of formation of  $P_1^*$ , which is  $k_1C_1$ , equals

$$
k_1 \times \frac{f_1 P_1}{b_1 + k_1} \times \frac{D_T}{1 + \frac{f_1 P_1}{b_1 + k_1} + \frac{f_2 P_2}{b_2 + k_2}}
$$
(5.10)

using Eq. [5.8](#page-48-0) and Eq. [5.9.](#page-48-1) We can thus write

$$
\frac{dP_1^*}{dt} = \frac{k_1 D_T P_1}{\frac{b_1 + k_1}{f_1} + P_1 + \frac{f_2(b_1 + k_1)}{f_1(b_2 + k_2)} P_2} \tag{5.11}
$$

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

$$
\frac{dP_2^*}{dt} = \frac{k_2 D_T P_2}{\frac{b_2 + k_2}{f_2} + P_2 + \frac{f_1(b_2 + k_2)}{f_2(b_1 + k_1)} P_1}.\tag{5.12}
$$

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 competitively inhibit the phosphorylation of the other by sequestering the enzyme DBT.

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

<span id="page-48-2"></span>
$$
\frac{dP_1^*}{dt} = \frac{V_1 P_1}{K + P_1 + P_2} \n\frac{dP_2^*}{dt} = \frac{V_2 P_2}{K + P_1 + P_2}
$$
\n(5.13)

with  $V_1 = k_1 D_T$  and  $V_2 = k_2 D_T$ . Eq. [5.13](#page-48-2) is the form used by Tyson *et al.* [\[15\]](#page-63-14).

#### <span id="page-49-0"></span>5.4.2 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 auto-regulation of per expression by PER dimers. The equation for per mRNA levels is then

<span id="page-49-4"></span>
$$
\frac{dM}{dt} = \frac{u}{1 + \left(\frac{P_2}{P_c}\right)^2} - d_M M\tag{5.14}
$$

with  $d_M$  being the rate of degradation of mRNA.

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

$$
\mathbf{P}_1 + \mathbf{P}_1 \xrightarrow[\phantom{a}b]{f} \mathbf{P}_2
$$

so that

<span id="page-49-2"></span>
$$
\frac{dP_1}{dt} = vM - \frac{V_1 P_1}{K + P_1 + P_2} - d_P P_1 - 2f P_1^2 + 2bP_2 \tag{5.15}
$$

using Eq. [5.13.](#page-48-2) Once phosphorylated, the PER monomers rapidly degrade and no longer contribute to the dynamics.

PER dimers also undergo phosphorylation, degradation, and monomerisation:

<span id="page-49-3"></span>
$$
\frac{dP_2}{dt} = -\frac{V_2 P_2}{K + P_1 + P_2} - d_P P_2 + f P_1^2 - b P_2.
$$
\n(5.16)

Once phosphorylated, the PER dimers degrade too.

By assuming equilibrum between PER monomers and dimers, Tyson *et al.* were able to reduce this system of three equations to two equations.

#### <span id="page-49-1"></span>5.4.3 Dimerisation

The dimerisation reaction of PER proteins is

$$
\mathrm{P}_1 + \mathrm{P}_1 \xrightarrow[\phantom{a}b]{f} \mathrm{P}_2
$$

and so at equilibrium

$$
P_2 = \frac{f}{b} P_1^2. \tag{5.17}
$$

If we write the total number of PER monomers, both free and in dimers, as  $P_T$ , where  $P_T$  can change with time, then

$$
P_T = P_1 + 2P_2 \tag{5.18}
$$

and so

$$
P_T = P_1 + 2\frac{f}{b}P_1^2\tag{5.19}
$$

which is a quadratic equation for  $P_1$ :

$$
P_1^2 + \frac{b}{2f}P_1 - \frac{b}{2f}P_T = 0.
$$
\n(5.20)

We can solve this equation following the usual formula

$$
P_1 = \frac{-1 + \sqrt{1 + 8\frac{f}{b}P_T}}{4\frac{f}{b}}
$$
  
= 
$$
\frac{2P_T}{1 + \sqrt{1 + 8\frac{f}{b}P_T}}
$$
(5.21)

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

<span id="page-50-1"></span>
$$
q = \frac{2}{1 + \sqrt{1 + 8\frac{f}{b}P_T}}.\t(5.22)
$$

Consequently the equilibrium concentrations both have a convenient form as functions of  $P_T$ :

<span id="page-50-2"></span>
$$
P_1 = qP_T \quad ; \quad P_2 = \frac{1}{2}(1-q)P_T \tag{5.23}
$$

with q given by Eq.  $5.22$ .

#### <span id="page-50-0"></span>5.4.4 The final model with two rate equations

By adding  $dP_1/dt$  to twice  $dP_2/dt$ , Tyson *et al.* find a differential equation for  $P_T = P_1 + 2P_2$ .

<span id="page-50-3"></span>
$$
\frac{dP_T}{dt} = vM - \frac{V_1q + V_2(1-q)}{K + \frac{1}{2}(1+q)P_T}P_T - d_P P_T
$$
\n(5.24)

using Eq. [5.23,](#page-50-2) which replaces Eq. [5.15](#page-49-2) and Eq. [5.16.](#page-49-3)

Similarly we use Eq. [5.23](#page-50-2) to write Eq. [5.14](#page-49-4) in terms of  $P_T$  rather than  $P_2$ :

<span id="page-50-4"></span>
$$
\frac{dM}{dt} = \frac{u}{1 + \frac{(1-q)^2 P_T^2}{4P_c^2}} - d_M M\tag{5.25}
$$

with q obeying Eq.  $5.22$ .

With now two variables,  $P_T$  and M, we can investigate the dynamics, Eq. [5.24](#page-50-3) and Eq. [5.25,](#page-50-4) using phase plane analysis. The nullclines intersect at one point, but this point is unstable for certain values of the parameters [\[15\]](#page-63-14), 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 cells must synthesise PER and then convert it into dimers before it represses transcription. The delayed negative feedback drives the circadian oscillations.

The system also has positive feedback. If the number of dimers is sufficiently high that the rate of phosphorylation of dimers by DBT saturates, 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 existing 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 monomers are more rapidly phosphorylated by DBT,  $V_2 \ll V_1$ . The positive feedback allows PER dimers to increase quickly once their numbers become sufficiently high.

#### <span id="page-51-0"></span>5.5 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' accumulating, and then a fast 'discharge', where the stress dissipates. The system must therefore have two widely separated time scales [\[14\]](#page-63-13).

The Tyson *et al.* model has positive and negative feedback and both slow and fast times scales. It exhibits relaxation oscillations. The increase of the numbers of PER dimers generates a slow time scale. This process is slow because mRNA must first by synthesised and then translated and because PER monomers are rapidly degraded by DBT  $(V_1 \gg V_2$  in Eq. [5.14](#page-49-4) and Eq. [5.15\)](#page-49-2). The decrease in the number of PER dimers once they repress transcription of per generates the fast time scale. The number of PER dimers quickly falls despite DBT preferentially degrading monomers because the loss of monomers causes the PER dimers to dissociate to maintain the dimer-monomer equilibrium.

#### <span id="page-51-1"></span>5.6 Oscillations through both positive and negative feedback

Oscillators with both positive and negative feedback are typically built around an underlying bistable system, although one that only 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 and has 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 via a component of the network regulated by light. They suggest the dissociation constant for PER's dimerisation [\[15\]](#page-63-14). 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 a hysteretic loop that would be generated by the positive feedback acting alone. Negative feedback prevents bistability and causes the system to oscillate, but the properties of the oscillations are still determined by the former hysteretic loop. The dynamics may be slow when the concentrations of the oscillating species are near the former steady-state values of the bistable system and will be fast when the concentrations of the oscillating species are moving between these values. The difference in concentrations between the two former steady states approximately determines the amplitude of the oscillations, and the time taken by the system to move around the loop determines their period.

Such relaxation oscillators can have an amplitude and frequency that are robust to stochastic fluctuations [\[16\]](#page-63-15). The amplitude is more robust because it is determined by the stable fixed points of the former bistability [\[16,](#page-63-15) [17\]](#page-63-16). The period is more robust at least to stochastic effects if the magnitude of the stochastic fluctuations tend to be higher when the oscillator is moving quickly. Then this noisy section of the oscillator's orbit is short-lived and contributes little to the period. Indeed, the circadian oscillator does moves faster when numbers of molecules are lower [\[18\]](#page-63-17).

The former stable steady states also allow dual feedback oscillators to tune the frequency of the oscillations while maintaining their amplitude [\[17\]](#page-63-16). Such behaviour is, for example, important for the effective functioning of the human heart. As the frequency changes, the former steady states need not. For most systems, however, the limit cycle of the oscillations does not completely follow the former hysteretic loop, and so the amplitude can change as the frequency changes. Usually this change is smaller, however, than the equivalent change in amplitude for an oscillator built from negative feedback alone [\[17\]](#page-63-16).

#### <span id="page-52-0"></span>5.6.1 Understanding a dual feedback oscillator

To understand better the role of the negative feedback in a dual feedback oscillator, consider a two-gene example: gene A is positively auto-regulated and activates a second gene B whose protein product represses gene A's transcription  $[19]$ . The auto-regulation generates positive feedback on gene A's expression; the repression through B generates negative feedback.



Modelling the positive feedback: Consider first the positive feedback. From Sec. [3.4,](#page-31-0) an equation describing the transcription of gene A is

<span id="page-52-1"></span>
$$
\frac{dM}{dt} = \frac{u_{\text{basal}} + u_{\text{max}} \frac{A^2}{K_2^2}}{1 + \frac{A^2}{K_A^2}} - d_M M \tag{5.26}
$$

where we include a basal rate and, to make things simpler, assume that a second protein  $A$  quickly binds the promoter once one binds, giving the denominator a term proportional to protein  $A^2$ but no term proportional to  $A$  — compare with Eq. [3.35.](#page-33-2) There is positive feedback because more protein A increase the rate of transcription causing more protein A to be synthesised. For translation, we again have Eq. [3.8,](#page-28-3)

<span id="page-52-2"></span>
$$
\frac{dA}{dt} = vM - d_A A. \tag{5.27}
$$

If the reactions controlling levels of mRNA are much faster than those controlling levels of protein, we can then approximate Eq. [5.26](#page-52-1) as being at quasi-steady state:  $dM/dt = 0$ . Solving for  $M$ , Eq. [5.27](#page-52-2) then becomes

<span id="page-52-3"></span>
$$
\frac{dA}{dt} = \frac{v}{d_M} \left[ \frac{u_{\text{basal}} + u_{\text{max}} \frac{A^2}{K_A^2}}{1 + \frac{A^2}{K_A^2}} \right] - d_A A. \tag{5.28}
$$

Simplifying through re-scaling: We will use re-scaling to reduce the number of parameters. There are two natural scales in the system — a time scale set by  $1/d_A$  and a concentration scale set by  $K_A$ . We re-scale by these two parameters to generate two dimensionless variables,  $t = d_A t$ and  $A = A/K_A$ . Dividing Eq. [5.28](#page-52-3) by  $K_A$  and by  $d_A$ , we can write

$$
\frac{1}{d_A} \cdot \frac{d}{dt} \left( \frac{A}{K_A} \right) = \frac{v}{d_A d_M K_A} \left[ \frac{u_{\text{basal}} + u_{\text{max}} \frac{A^2}{K_A^2}}{1 + \frac{A^2}{K_A^2}} \right] - \frac{A}{K_A} \tag{5.29}
$$

or

$$
\frac{d\tilde{A}}{d\tilde{t}} = \frac{v}{d_A d_M K_A} \left[ \frac{u_{\text{basal}} + u_{\text{max}} \tilde{A}^2}{1 + \tilde{A}^2} \right] - \tilde{A}.
$$
 (5.30)

Finally we will define  $\alpha = \frac{v u_{\text{max}}}{d_A d_B K}$  $\frac{vu_{\text{max}}}{d_A d_M K_A}$  and  $b = u_{\text{basal}}/u_{\text{max}}$  giving

<span id="page-53-0"></span>
$$
\frac{d\tilde{A}}{d\tilde{t}} = \alpha \left[ \frac{b + \tilde{A}^2}{1 + \tilde{A}^2} \right] - \tilde{A}.
$$
\n(5.31)

Comparing Eq. [5.31](#page-53-0) and Eq. [5.28,](#page-52-3) the rescaling has decreased the number of parameters from six to two.

Adding negative feedback: To include negative feedback on gene A, we will let protein A activate gene B, and protein  $B$  repress gene A. No longer explicitly writing the tildes, but time is still in units of  $1/d_A$  and concentration in units of  $K_A$ , we can write [\[19\]](#page-64-0)

<span id="page-53-1"></span>
$$
\frac{dA}{dt} = \frac{\alpha \left[b + A^2\right]}{\left[1 + \left(\frac{B}{K}\right)^2\right] \left[1 + A^2\right]} - A
$$
\n
$$
\frac{dB}{dt} = \kappa A - d_B B.
$$
\n(5.32)

There are two simplifying assumptions. First, the binding sites of  $A$  and  $B$  are sufficiently far apart on the promoter of gene A that the two proteins do not interact: the equivalent of  $K_i$  in Eq. [3.38](#page-34-1) is one so that the denominator in Eq. [5.32](#page-53-1) factorises. Second, the rate of transcription of gene B is simply proportional to A: we model transcription just as in Eq. [3.23,](#page-31-4) but impose  $A \ll K_1$ .

**Bistability for fixed B:** Fixing  $B$  at a particular concentration, we'll show is equivalent to changing the value of  $\alpha$  in Eq. [5.32.](#page-53-1) At steady state,  $dA/dt = 0$  and so

<span id="page-53-2"></span>
$$
\tilde{\alpha}(b + A^2) = A(1 + A^2) \tag{5.33}
$$

where

$$
\tilde{\alpha} = \frac{\alpha}{\left[1 + \left(\frac{B}{K}\right)^2\right]}.\tag{5.34}
$$

Rearranging Eq. [5.33](#page-53-2) gives

<span id="page-53-3"></span>
$$
A^3 - \tilde{\alpha}A^2 + A - \tilde{\alpha}b = 0,\tag{5.35}
$$

a cubic equation.

We will use Descartes's rule of signs to determine the number of positive solutions of Eq. [5.35.](#page-53-3) For a polynomial equation

$$
x^{n} + a_{n-1}x^{n-1} + a_{n-2}x^{n-2} + \dots + a_{1}x + a_{0} = 0,
$$
\n(5.36)

Descartes showed that the maximum number of positive roots is equal to the number of changes of sign in the polynomial's coefficients moving from left to right  $[20]$ . Further, if there are N changes of sign, the number of positive routes is either N or  $N-2$  or  $N-4$ , etc. For Eq. [5.35,](#page-53-3) we have three changes of sign and so either three positive roots – bistability – or one positive root – monostability (Fig. [14\)](#page-54-0).

<span id="page-54-0"></span>

Figure 14: For intermediate, fixed values of B, the positive feedback generates two stable steady-state solutions for A. By changing B, we change  $\tilde{\alpha}$  and so the solutions of Eq. [5.35.](#page-53-3) Here  $\alpha = 50$ ,  $b = 0.01$ ,  $K = 0.02$ ,  $d_B = 0.01$ , and  $\kappa = 0.8d_B$ .

A limit cycle when B is not fixed: How B causes oscillations by de-stabilising A is easiest to understand when levels of B change slowly compared to levels of A [\[19\]](#page-64-0). Let  $d_B \ll 1$  in Eq. [5.32](#page-53-1) and let  $\kappa$ , which determines the time scale of B's synthesis, be of the same size as  $d_B$ :  $\kappa = \mathcal{O}(d_B)$ . For example,  $\kappa = 0.8d_B$  in Fig. [14.](#page-54-0) Then B responds slowly to changes in A, which moves quickly in comparison.

To generate oscillations in an anticlockwise direction around the bistable solutions in Fig. [14,](#page-54-0) we wish  $B$  to destabilise  $A$  when  $A$  is at the lower limit of the left branch in Fig. [14.](#page-54-0)  $A$  will then jump to the right branch. Similarly,  $B$  should also destabilise  $A$  when  $A$  reaches the upper limit of the right branch so that A jumps back to the left one.

When A is at the left branch's lower limit, the magnitude of the system's negative feedback should therefore be decreasing so that A's rate of synthesis grows, favouring A moving to the right branch with its higher levels of A. To have decreasing negative feedback, levels of B should be falling so that there is less repression. Therefore we require  $dB/dt < 0$  when A is at the left branch's lower limit.

When A is at the right branch's upper limit, the system's negative feedback should be increasing so that  $A$ 's rate of synthesis diminishes, favouring  $A$  moving to the left branch with its lower levels of A. Therefore we require  $dB/dt > 0$  when A is at the right branch's upper limit so that levels of B are rising, generating more repression.

One way to impose these two conditions is to have the nullcline of B, where  $dB/dt = 0$ , pass between the two bistable solutions (Fig. [15A](#page-55-0)). To the nullcline's left, for smaller A,  $dB/dt < 0$ from Eq. [5.32,](#page-53-1) and, to the nullcline's right, for larger A,  $dB/dt > 0$ , as we require. The negative feedback then destabilises the steady-state solutions, generating oscillations (Fig. [15A](#page-55-0)). If the nullcline intercepts the bistable solutions, however, there are no oscillations (Fig. [15B](#page-55-0)).

When A is near the former low steady states, the system moves slowly. There positive feedback is weak, and  $A$ 's synthesis rate is only slowly increasing as  $B$  slowly decreases. Eventually there is insufficient  $B$  to repress gene A, and levels of  $A$  quickly increase through positive feedback with A spiking and moving near the former high steady states. The now slowly increasing B and the high degradation rate of A quickly decrease levels of A. When levels are low enough

to weaken the positive feedback and  $B$  is sufficiently high to repress gene A,  $A$  moves quickly back to near the former low steady states. The positive feedback is then again weak and the negative feedback is strong because of the high levels of B. Levels of A change slowly once more.

<span id="page-55-0"></span>

Figure 15: The negative feedback generates oscillations by destabilising the steady states. Here  $\alpha = 50$ ,  $b = 0.01, K = 0.02$ , and  $d_B = 0.01$ , as before. **A** When  $\kappa = 0.8d_B$ , the nullcline of B passes between the branches of stable steady states that exist when  $B$  is fixed. The negative feedback therefore encourages A to jump from the left to the right branch when A and B are small and from the right to the left branch when  $A$  and  $B$  are large. The systems oscillates. The inset shows the limit cycle generated by the simulated time series in blue: with  $d_B \ll 1$ , the oscillations are around the former steady states generated by the positive feedback. **B** When  $\kappa = 5d_B$ , the nullcline of B does not pass between the two branches. At the lower limit of the left branch, when A and B are both small, B is increasing and so too is the magnitude of the negative feedback. A's rate of synthesis is therefore falling, favouring A remaining near the former steady state with its low values of A. There are no oscillations.

We can understand too some of the properties of the oscillations [\[19\]](#page-64-0). The difference in time scales describing A and B's dynamics imposed by  $d_B \ll 1$ , or if we remove the re-scaling by  $d_B \ll d_A$ , generates relation oscillations. Levels of B change slowly, but levels of A spike when A quickly moves from near the former low steady states to near the former high steady states and then is rapidly degraded. The slow dynamics of  $B$  means that  $B$  principally determines the period, which increases as the time scale associated with B increases — when  $d_B$  decreases. The positive feedback determines the amplitude of the oscillations through the values of A at the former steady states: the size of the spikes in  $A$  is proportional to the distance between these steady states.

# <span id="page-57-0"></span>Appendix A Simulating stochastic biochemical reactions

Often we use the Gillespie algorithm [\[1\]](#page-63-0) to simulate fluctuations in biochemical systems. The computer "rolls" the equivalent of two dice: one to choose which reaction will occur next and the other to choose when that reaction will occur.

Consider a system in which  $n$  different reactions are possible, then we should first calculate the probability that each type of reaction will occur at the end of an interval of time  $t$ . Let this probability per unit time be  $P_i(t)$  for reaction i. For example, if reaction i corresponds to the second-order reaction

$$
A + B \xrightarrow{\tilde{f}} C
$$

then

<span id="page-57-1"></span>P(reaction *i* in an interval  $\delta t$ ) =  $N_A N_B \tilde{f} \delta t$  $\equiv a_i \delta t$ 

where  $a_i = N_A N_B \tilde{f}$  is referred to as reaction *i*'s propensity and *ot* is sufficiently small that only one reaction can occur. We can use Eq. [A.1](#page-57-1) to split the probability  $P_i(t)\delta t$  into two events:

$$
P_i(t)\delta t = P(\text{no reactions for time } t) \times P(\text{reaction } i \text{ in the interval } \delta t)
$$
 (A.1)

and if we write  $P_0(t)$  as the probability of no reactions occurring during an interval t, then

<span id="page-57-2"></span>
$$
P_i(t)\delta t = P_0(t)a_i\delta t. \tag{A.2}
$$

To find  $P_0(t)$ , consider the probability of having no reactions during an interval  $t+\delta t$ , which is the product of the probability of having no reactions during  $t$  and the probability of no reactions occurring during  $\delta t$ :

$$
P_0(t + \delta t) = P_0(t) \left[ 1 - \sum_{j=1}^{n} a_j \delta t \right]
$$
 (A.3)

where the sum runs over the propensities for all the reactions. Letting  $\delta t$  go to zero implies that

$$
\frac{dP_0}{dt} = -P_0 \sum_{j=1}^{n} a_j \tag{A.4}
$$

and so

<span id="page-57-3"></span>
$$
P_0(t) = \mathcal{N} \exp\left(-t \sum_{j=1}^n a_j\right)
$$
 (A.5)

where  $\mathcal N$  is a normalisation constant that ensures  $\int_0^\infty P_0(t)dt = 1$ . Thus we have

$$
P_i(t) = \mathcal{N} a_i e^{-t \sum_j a_j} \tag{A.6}
$$

from Eq. [A.2](#page-57-2) and where  $\mathcal N$  is chosen so that  $\int_0^\infty P_1(t)dt = 1$ .

In practice, to choose which reaction to simulate, an  $n$ -sided die is rolled with each side corresponding to a reaction and weighted by that reaction's propensity. A second die is then used to determine the time when the reaction occurs by sampling from Eq. [A.5.](#page-57-3) All the chemical species and the time variable are updated following the chosen reaction. For example, if reaction i is chosen then the number of A and B molecules are both decreased by one and the number of C molecules is increased by one, and the propensities of all the reactions are correspondingly recalculated. The process is then repeated, randomly picking both a new reaction and when it happens.

#### <span id="page-58-0"></span>A.1 Mesoscopic and macroscopic rates

The correct way to interpret rate constants is as probabilities per unit time. This interpretation is consistent with the macroscopic one where rates are considered as the reciprocal of the average time taken for the reaction to occur.

For example, consider a system with only one molecule of A and one molecule of B that undergo

$$
A + B \xrightarrow{\tilde{f}} C
$$

where  $\tilde{f}$  is the probability of a pair of molecules reacting in unit time. This reaction's propensity will be  $a = \tilde{f} \times 1 \times 1$ , and, from Eq. [A.5,](#page-57-3) the time taken for the pair to react is given by

$$
P_0(t) = ae^{-at} = \tilde{f}e^{-\tilde{f}t}
$$
\n(A.7)

which satisfies  $\int_0^\infty P_0(t)dt = 1$ . We can then calculate the mean time for the reaction

$$
\bar{t} = \int_0^\infty t \tilde{f} e^{-\tilde{f}t} dt = \tilde{f}^{-1}
$$
\n(A.8)

and so we are able to interpret  $\tilde{f}$  too as the reciprocal of the mean time for a pair of molecules to react.

# <span id="page-58-1"></span>Appendix B 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

<span id="page-58-2"></span>
$$
\frac{dA}{dt} = -kA\tag{B.1}
$$

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 [\[21\]](#page-64-2).

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  $\vert$  is read as 'given') [\[21\]](#page-64-2)

$$
P(k|D) = \frac{P(D|k)P(k)}{P(D)}
$$
  
 
$$
\propto P(D|k)P(k)
$$
 (B.2)

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

<span id="page-59-4"></span>
$$
P(k, A_0|D) \propto P(D|k, A_0)P(k, A_0)
$$
\n(B.3)

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,  $\epsilon$ , is assumed to be identically, independently, and normally distributed with a mean of zero and a standard deviation  $\sigma$ , which determines its typical size:

<span id="page-59-0"></span>
$$
P_e(\epsilon) = \frac{\exp\left(\frac{-\epsilon^2}{2\sigma^2}\right)}{\sqrt{2\pi}\sigma} \tag{B.4}
$$

If  $d_i$  is the data point measured at time  $t_i$  and  $A_i$  is the corresponding predicted value, found by integrating Eq [B.1,](#page-58-2) then  $d_i$  and  $A_i$  are related through the measurement error at that time point,  $\epsilon_i$ :

$$
d_i = A_i + \epsilon_i \tag{B.5}
$$

or

$$
d_i - A_i = \epsilon_i. \tag{B.6}
$$

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 [B.1\)](#page-58-2). Using Eq [B.4,](#page-59-0) we can write

<span id="page-59-1"></span>
$$
P(d_i|k, A_0) = P(d_i|A_i)
$$
  
=  $P_e(d_i - A_i) = \frac{\exp\left(\frac{-(d_i - A_i)^2}{2\sigma^2}\right)}{\sqrt{2\pi}\sigma}$  (B.7)

assuming that the value of  $\sigma$  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

<span id="page-59-2"></span>
$$
P(D|k, A_0) = P(d_0, ..., d_n|k, A_0)
$$
  
=  $P(d_0, ..., d_n|A_0, A_1, ..., A_n)$   
=  $P(\epsilon_i, ..., \epsilon_n)$   
=  $P_e(\epsilon_i) ... P_e(\epsilon_n)$  (B.8)

where we assume *n* data points. Using Eq [B.7,](#page-59-1) Eq [B.8](#page-59-2) is

<span id="page-59-3"></span>
$$
P(D|k, A_0) = \prod_{i=0}^{i=n-1} \frac{\exp\left(\frac{-(d_i - A_i)^2}{2\sigma^2}\right)}{\sqrt{2\pi}\sigma} \\ = \frac{\exp\left(-\sum_{i=0}^{n-1} \frac{-(d_i - A_i)^2}{2\sigma^2}\right)}{\left(\sqrt{2\pi}\sigma\right)^n}
$$
(B.9)

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 [B.9](#page-59-3) with the prior distribution,  $P(k, A_0)$ , and Eq [B.3](#page-59-4) allows the posterior probability of k and  $A_0$  to be calculated. Figs. [16](#page-61-0) and [17](#page-62-0) 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 store accurately).

First, typically the negative logarithm of the likelihood is calculated. Taking the logarithm of Eq [B.9,](#page-59-3) this 'energy', as it is sometimes called in analogy with approaches from physics, is

$$
\mathcal{E} = \sum_{i=0}^{n-1} \frac{(d_i - A_i)^2}{2\sigma^2} + n \log(\sqrt{2\pi}) + n \log(\sigma)
$$
 (B.10)

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

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 maximise the posterior probability or, equivalently for suitable prior distributions, minimise the energy. Parameter values are typically positive in systems biology, but numerical optimisation schemes may not allow this bound to be imposed. A trick is to transform the parameters

<span id="page-60-0"></span>
$$
k = \exp(\tilde{k}) \quad ; \quad A_0 = \exp(\tilde{A}_0) \tag{B.11}
$$

and minimise the energy as a function of  $\tilde{k}$  and  $\tilde{A}_0$ . If the optimisation causes either of these transformed parameters to become negative, then k and  $A_0$  are still positive from Eq [B.11.](#page-60-0)

<span id="page-61-0"></span>

Figure 16: 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.

<span id="page-62-0"></span>

Figure 17: 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.

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