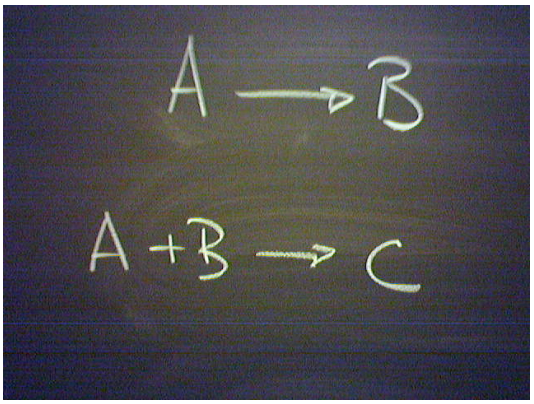

Figure 1.7: Two possible chemical reactions.

1.2 Boxes and arrows to differential equations

When we draw a picture such as Fig 1.7 to describe a chemical reaction, we could mean one of two things. First, we could simply be stating the fact that, through an unspecified process, substance A turns into substance B, and similarly in some other process A and B combine to make C. For example, if this process involves a catalyst, it could be that the rate of the reaction controlled by the availability of the catalyst and thus has nothing to do with the concentration of A and B molecules.

A second interpretation is that this figure really describes the *mechanism* of the reactions. Then $A \rightarrow B$ means that the conversion of A into B is a *first order* reaction. Intuitively, a first order reaction is one in which each molecule makes an independent decision about whether to complete the reaction, not depending on encounters with any other molecule. If this is the case, then the number of B molecules which are created must be proportional to the number of A molecules that are available to react. It is conventional for most of chemistry to talk not about the number of molecules, but about their concentration or number per unit volume. If we write the concentration of species i as $C_{\rm i}$, then for our simple first order reaction we have

$$\frac{dC_B}{dt} = kC_A, \tag{1.75}$$

where k is the *first order rate constant*. Note that sometimes one writes $[A]$ to denote the concentration of A. It is important to get used to different notations, as long as they are used consistently within each argument! C_A and C_B have the same units, so in order for Eq (1.75) to make sense, k has to have units of 1/time, conventionally 1/s.

If A is turning into B, then each molecule of B which appears must correspond to a molecule of A which disappeared. Thus we have to have

$$\frac{dC_A}{dt} = -kC_A. \tag{1.76}$$

We've seen this equation before, since it is the same as for the velocity of a particle moving through a viscous fluid, assuming that the drag is proportional to velocity. So we know the solution:

$$C_A(t) = C_A(0)e^{-kt}. \tag{1.77}$$

Then we can also solve for C_B:

$$\begin{aligned}
\frac{dC_B}{dt} &= kC_A \\
&= kC_A(0)e^{-kt} && (1.78)\\
\int_0^t dt\,\frac{dC_B}{dt} &= \int_0^t dt\,kC_A(0)e^{-kt} && (1.79)\\
C_B(t) - C_B(0) &= kC_A(0)\int_0^t dt\,e^{-kt} && (1.80)\\
&= kC_A(0)\left[-\frac{1}{k}e^{-kt}\right]\Bigg|_{t=0}^{t} && (1.81)\\
&= kC_A(0)\left[-\frac{1}{k}e^{-kt} + \frac{1}{k}\right] && (1.82)\\
&= C_A(0)[1 - e^{-kt}] && (1.83)\\
C_B(t) &= C_B(0) + C_A(0)[1 - e^{-kt}]. && (1.84)
\end{aligned}$$

So we see that C_A decays exponentially to zero, while C_B rises exponentially to its steady state; as an example see Fig 1.8. One of the great examples of a first order reaction is radioactive decay, and this is why the abundance of unstable isotopes (e.g., ^{14}C, ^{235}U, ...) in a sample decays exponentially, and this will be very important in the next section.

Problem 12: Just to be sure that you understand first order kinetics ... If the half life of a substance that decays via first order kinetics is $t_{1/2}$, how long do you have to wait until 95% of the initial material has decayed? Explain why this question wouldn't make sense in the case of second order kinetics.

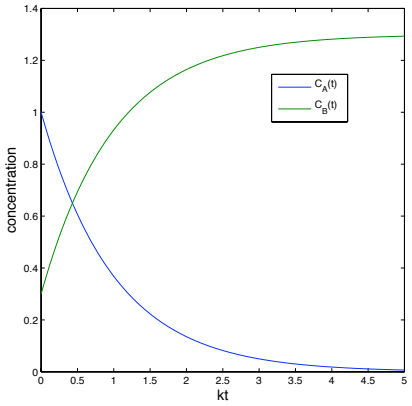

Figure 1.8: Dynamics of the concentrations in a first order reaction $A \rightarrow B$.

What about the case $A + B \rightarrow C$? If we take this literally as a mechanism, we are describing a *second order* reaction, which means that A and B molecules have to find each other in order to make C. The rate at which C molecules are made should thus be proportional to the rate at which these pairwise encounters are happening. What is this rate? If you imagine people milling around at random in a large room, it's clear that the number of times per second that people run into each other depends both on how many people there are and on the size of the room. If you follow one person, the rate at which they run into people should go up if there are more people, and down if the room gets bigger. Plausibly, what matters is the density of people—the number of people divided by the size of the room—which is just like measuring the concentration of molecules.

To obtain the rate of a second order reaction $A+B \rightarrow C$, we thus need to count the rate at which A molecules bump into B molecules as they wander around randomly. By analogy with the people milling around the room, if we follow one A molecule, the rate at which it bumps into B molecules will be proportional to the concentration of B molecules. But then the total rate of encounters between A and B will be proportional to the number of A molecules multiplied by the concentration of B, so if we measure the number of encounters per unit volume per second, we'll get an answer proportional to the product of the concentrations of A and B. Thus,

$$\frac{d[C]}{dt} = k_2[A][B]. \tag{1.85}$$

Corresponding the to formation of C is the destruction of both A and B, so

we must have

$$\frac{d[A]}{dt} = -k_2[A][B] \tag{1.86}$$

$$\frac{d[B]}{dt} = -k_2[A][B]. \tag{1.87}$$

The rate constant k_2 is now a *second order rate constant*, and you can see that it has different units from the first order rate constant k in the equations above; $k_2 \sim 1/(\text{time} \cdot \text{concentration})$, conventionally $1/(\text{M} \cdot \text{s})$.

Perhaps the simplest second order reaction is $A + A \rightarrow B$, for which the relevant equations are

$$\frac{d[A]}{dt} = -k_2[A]^2 \tag{1.88}$$

$$\frac{d[B]}{dt} = k_2[A]^2. \tag{1.89}$$

We have sen this equation before, describing the velocity of a particle that experiences drag proportional to velocity squared. Thus we can proceed as we did before:

$$\frac{d[A]}{dt} = -k_2[A]^2$$

$$\frac{d[A]}{[A]^2} = -k_2 dt \tag{1.90}$$

$$\int_{[A]_0}^{[A]_t} \frac{d[A]}{[A]^2} = k_2 \int_0^t dt \tag{1.91}$$

$$-\frac{1}{[A]}\Big|_{[A]_0}^{[A]_t} = -k_2 t \tag{1.92}$$

$$-\frac{1}{[A]_t} + \frac{1}{[A]_0} = -k_2 t \tag{1.93}$$

$$\frac{1}{[A]_0} + k_2 t = \frac{1}{[A]_t} \tag{1.94}$$

$$[A]_t = \frac{[A]_0}{1 + k_2[A]_0 t}, \tag{1.95}$$

where $[A]_t$ is the concentration of A at time t, and in particular $[A]_0$ is the concentration when $t = 0$. Thus the initial concentration does not decay as an exponential, but rather as $\sim 1/t$ at long times; the time for decay to half the initial value is $t_{1/2} = 1/(k_2[A]_0)$ and depends on the initial concentration. Notice that

$$[A]_{t \gg t_{1/2}} \approx \frac{[A]_0}{k_2[A]_0 t} = \frac{1}{k_2 t}, \tag{1.96}$$

so that after a while the concentration is still changing, but the amount of stuff we have left is independent of how much we started with (!).

Problem 13: Check that you understand each of the steps leading to Eq (1.95). As a test of your understanding, consider the (rather unusual) case of a third order reaction, in which three A molecules come together to react irreversibly. This is described by

$$\frac{d[A]}{dt} = -k_3[A]^3. \tag{1.97}$$

What are the units of the third order rate constant k_3? Can you solve this equation?

time t (minutes)	$[A]/[A]_0$	$[B]/[B]_0$
0.25	0.7157	0.7635
0.50	0.7189	0.4305
0.75	0.5562	0.5262
1.00	0.4761	0.6195
1.25	0.4948	0.4876
1.50	0.3096	0.3169
1.75	0.3842	0.3702
2.00	0.2022	0.2764
2.25	0.1872	0.2613
2.50	0.1971	0.2738

Table 1.1: Two kinetics experiments.

Problem 14: Imagine that you do two experiments in chemical kinetics. In one case we watch the decay of concentration is some reactant A, and in the other case the reactant is B. The half lives of both species are about one minute, and perhaps because you are in a hurry you run the reactions out only for 2.5 minutes. You take samples of the concentration every quarter of a minute, and you get the results in Table 1.1. Perhaps the first thing you notice is that the concentrations don't decrease monotonically with time. Presumably this is the result of errors in the measurement.

(a.) Can you decide whether the reactions leading to the decay of A and B are first order or second order? Are A and B decaying in the same way, or are they different?

(b.) Other than making more accurate measurements, how could you extend these experiments to give you a better chance at deciding if the reactions are first or second order?

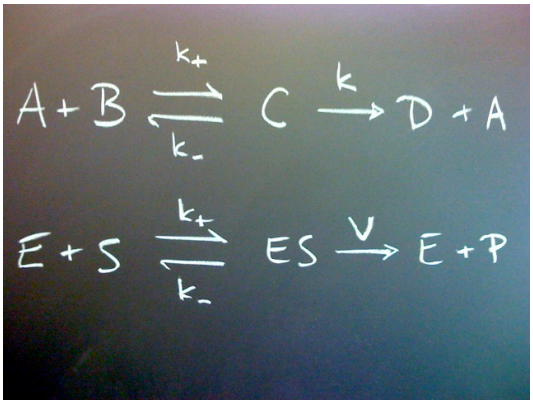

Figure 1.9: At the top, a reaction scheme for the conversion of B into D, using A as a catalyst. At the bottom, this scheme is written to make better connections with the idea of catalysis by an enzyme: E is the enzyme, S is the 'substrate' that gets converted into the product P, and ES is a complex of the enzyme and substrate bound to one another. The rate constants $k_\pm$ have the same notation, but we write the rate for $ES \to E+P$ as V, since it's the 'velocity' of the enzyme.

An important point about all this is that when we draw a more complex reaction mechanism, each and every arrow corresponds to a term in the differential equation, and the sign of the term depends on the direction of the arrow. Consider, for example, the reactions shown at the top of Fig 1.9. Really this is a scheme in which B is converted into D, and the A molecules participate but are not consumed: the A molecules are catalysts. Notice that there are three separate reactions, one for each arrow: $A + B \to C$, which occurs with a second order rate constant k_+, $C \to A + B$, which occurs with first order rate k_-, and $C \to D + A$, with first order rate k. We write k_+ and k_- because these are forward and reverse processes.

Now we have to write out the differential equations, using the rule that each reaction or arrow generates its corresponding term. Probably it's easiest to start with the equation for $[C]$, since all three reactions contribute. We see the arrow coming "in" to C from the left, which corresponds to the concentration of C changing at a rate $k_+[A][B]$. There is a second arrow at the left, which corresponds to the concentration of C changing at a rate $-k_-[C]$, where the negative sign is because the arrow points "out" and describes the destruction of C molecules. Finally, there is an arrow point out to the right, which corresponds to the concentration of C changing at a rate $-k[C]$. Putting all of these terms together, we have

$$\frac{d[C]}{dt} = +k_+[A][B] - k_-[C] - k[C]. \tag{1.98}$$

For $[B]$, only the k_+ and k_- processes contribute:

$$\frac{d[B]}{dt} = -k_+[A][B] + k_-[C]. \tag{1.99}$$

Finally, for $[A]$, all three reactions contribute, but with the opposite signs from Eq (1.98):

$$\frac{d[A]}{dt} = -k_+[A][B] + k_-[C] + k[C]. \tag{1.100}$$

The important point here is not to solve these equations (yet), but rather to be sure that you understand how to go from the pictures with arrows describing the reactions down to the equations that describe quantitatively the dynamics of the concentrations.

Notice that in Fig 1.9 we have also rewritten the scheme to make clear that it describes an enzyme which converts 'substrates' S into 'products' P. In fact this is one of the standard schemes for describing biochemical reactions, and it's called Michaelis–Menten kinetics. To make contact with the standard discussion, let's call the concentration of substrates $[S]$, the concentration of products $[P]$, and so on. Then the kinetic equations become

$$\begin{aligned}
\frac{d[S]}{dt} &= -k_+[S][E] + k_-[ES] && (1.101)\\
\frac{d[ES]}{dt} &= k_+[S][E] - (k_- + V)[ES] && (1.102)\\
\frac{d[P]}{dt} &= V[ES] && (1.103)\\
\frac{d[E]}{dt} &= -k_+[S][E] + (k_- + V)[ES]. && (1.104)
\end{aligned}$$

You should notice that Eq's (1.102) and (1.104) can be combined to tell us that

$$\frac{d([ES] + [E])}{dt} = 0, \tag{1.105}$$

or equivalently that $[ES] + [E] = [E]_0$, the total enzyme concentration. Solving all these equations is hard, but there is an approximation in which everything simplifies.

Suppose that there is a lot of the substrate, but relatively little enzyme. Then the high concentration of the substrate means that the binding of the substrate to the enzyme will be fast. Although this isn't completely obvious, one consequence is that the concentration of the enzyme–substrate complex

ES will come very quickly to a steady state; in particular this steady state will be reached before the substrate concentration has a chance to change very much. But we can find this steady state just by setting $d[ES]/dt = 0$ in Eq (1.102):

$$\begin{aligned} 0 = \frac{d[ES]}{dt} &= k_+[S][E] - (k_- + V)[ES] && (1.106)\\ \Rightarrow 0 &= k_+[S][E] - (k_- + V)[ES] && (1.107)\\ k_+[S][E] &= (k_- + V)[ES]. && (1.108) \end{aligned}$$

Now we use the constancy of the total enzyme concentration, $[ES] + [E] = [E]_0$, to write $[E] = [E]_0 - [ES]$, and substitute to solve for $[ES]$:

$$\begin{aligned} k_+[S][E] &= (k_- + V)[ES] && \\ k_+[S]([E]_0 - [ES]) &= (k_- + V)[ES] && (1.109)\\ k_+[S][E]_0 - k_+[S][ES] &= (k_- + V)[ES] && (1.110)\\ k_+[S][E]_0 &= (k_- + V)[ES] + k_+[S][ES] && (1.111)\\ &= (k_- + V + k_+[S])[ES] && (1.112)\\ \frac{k_+[S][E]_0}{k_- + V + k_+[S]} &= [ES]. && (1.113) \end{aligned}$$

The reason it is so useful to solve for $[ES]$ is that, from Eq (1.103), the rate at which product is formed is just $V[ES]$, so we find

$$\frac{d[P]}{dt} = V[E]_0 \frac{k_+[S]}{k_- + V + k_+[S]}, \tag{1.114}$$

or

$$\frac{d[P]}{dt} = V[E]_0 \frac{[S]}{[S] + K_m}, \tag{1.115}$$

where $K_m = (k_- + V)/k_+$ is sometimes called the Michaelis constant.

Equation (1.115) is the main result of Michaelis–Menten kinetics, and it is widely used to describe real enzymes as they catalyze all sorts of reactions inside cells. What is this formula telling us? To begin, the rate at which we make product is proportional to the concentration of enzymes. Although we have written equations for the macroscopic concentration of molecules, we can think of this in terms of what individual molecules are doing: each enzyme molecule can turn substrate into product at some rate, and the total rate is then this 'single molecule' rate multiplied by the total number of enzyme molecules. In addition, Eq (1.115) tells us that is the

substrate concentration is really low ($[S] \ll K_m$), then the rate at which catalysis happens is proportional to how much substrate we have; on the other hand once the substrate concentration is large enough ($[S] \gg K_m$), finding substrate molecules is not the problem and the rate of catalysis is limited by the properties of the enzyme itself (V).

Problem 15: The enzyme lysozyme helps to break down complex molecules built out of sugars. As a first step, these molecules (which we will call S) must bind to the enzyme. In the simplest model, this binding occurs in one step, a second order reaction between the enzyme E and the substrate S to form the complex ES:

$$E + S \stackrel{k_+}{\rightarrow} ES, \tag{1.116}$$

where k_+ is the second order rate constant. The binding is reversible, so there is also a first order process whereby the complex decays into its component parts:

$$ES \stackrel{k_-}{\rightarrow} E + S, \tag{1.117}$$

where k_- is a first order rate constant. Let's assume that everything else which happens is slow, so we can analyze just this binding/unbinding reaction.

(a.) Write out the differential equations that describe the concentrations of $[S]$, $[E]$ and $[ES]$. Remember that there are contributions from both reactions (1.116) and (1.117).

(b.) Show that if we start with an initial concentration of enzyme $[E]_0$ and zero concentration of the complex ($[ES]_0 = 0$), then there is a conservation law: $[E] + [ES] = [E]_0$ at all times.

(c.) Assume that the initial concentration of substrate $[S]_0$ is in vast excess, so that we can always approximate $[S] \approx [S]_0$. Show that there is a steady state at which the concentration of the complex is no longer changing, and that at this steady state

$$[ES]_{\rm ss} = [E]_0 \cdot \frac{[S]}{[S] + K}, \tag{1.118}$$

where K is a constant. How is K related to the rate constants k_+ and k_-?

(d.) When the substrate is (N–acetylglucosamine)$_2$, experiments near neutral pH and at body temperature show that the rate constants are $k_+ = 4 \times 10^7\,{\rm M}^{-1}{\rm s}^{-1}$ and $k_- = 1 \times 10^5\,{\rm s}^{-1}$. What is the value of the constant K [in Eq (1.118)] for this substrate? At a substrate concentration of $[S] = 1\,{\rm mM}$, what fraction of the initial enzyme concentration will be in the the complex $[ES]$ once we reach steady state?

(e.) Show that the concentration of the complex $[ES]$ approaches its steady state exponentially: $[ES](t) = [ES]_{\rm ss}[1 - \exp(-t/\tau)]$. Remember that we start with $[ES]_0 = 0$. How is the time constant τ related to the rate constants k_+ and k_- and to the substrate concentration $[S]$? For (N–acetylglucosamine)$_2$, what is the *longest* time τ that we will find for the approach to steady state?

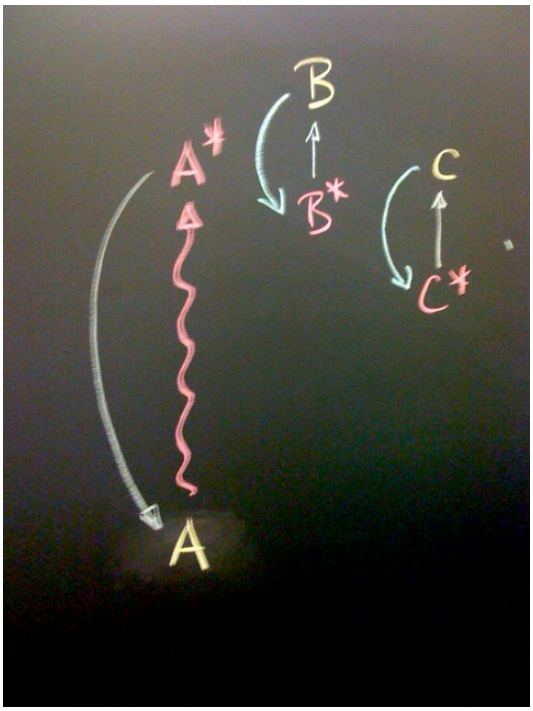

Figure 1.10: A cascade of enzymatic reactions.

Another interesting example is a sequence or cascade of reactions, as schematized in Fig 1.10. Here we imagine that there is a molecule A which can be stimulated by some signal to go into an activated states A^*. Once in this activated state, it can act as a catalyst, converting B molecules into their activated state B^*. The active B^* molecules act as a catalyst for C, and so on. This sort of scheme is quite common in biological systems, and serves as a molecular amplifier—even if we activate just one molecule of A, we can end up with many molecules at the output of such a cascade.

One example that we should keep in mind is happening in the photoreceptor cells of your retina as you read this. In these cells, the A molecules are rhodopsin, and the stimulation is what happens when these molecules absorb light. Once rhodospin is in an active state, it can catalyze the activation of the B molecules, which are called transducin. Transducin is one member of a large family of proteins (called G–proteins) that are involved in many different kinds of signaling and amplification in all cells, not just vision. The C molecules are enzyme called phosphodiesterase, which chew up molecules of cyclic GMP (cGMP, which would be D if we continued our schematic). Again, lots of cellular processes use cyclic nucleotides (cGMP and cAMP) as internal signals or 'second messengers' in cells. In the pho-

toreceptors, cGMP binds to proteins in the cell membrane that open holes in the membrane, and this allows the flow of electrical current; more about this later in the course. These electrical signals get transmitted to other cells in the retina, eventually reaching the cells that form the optic nerve and carry information from the eye to your brain.

How can we describe the dynamics of a cascade such as Fig 1.10? Let's think about the way in which $[B^*]$ changes with time. We have the idea that A^* catalyzes the conversion of B into B^*, so the simplest possibility is that this is a second order process: the rate at which B^* is produced will be proportional both to the amount of A^* and to the number of available B molecules, with a second order rate constant k_2. Presumably there is also a back reaction so that B^* converts back into B at some rate k_-. Then the dynamics are described by

$$\frac{d[B^*]}{dt} = k[A^*][B] - k_-[B^*]. \tag{1.119}$$

There must be something similar for the way in which C^* is formed by the interaction of B^* with C, and for simplicity let's assume that all the rate constants are the same (this doesn't matter for the point we want to make here!):

$$\frac{d[C^*]}{dt} = k[B^*][C] - k_-[C^*]. \tag{1.120}$$

Actually solving these equations isn't so simple. But let's think about what happens at very early times. In Eq (1.119), we can assume that at $t = 0$ we start with none of the activated B^*. The external stimulus (e.g., a flash of light to the retina) comes along and suddenly we have lots of A^*. There's plenty of B around to convert, and so there is an initial rate $k[A^*]_0[B]_0$, which means that the number of activated B molecules will grow

$$[B^*] \approx k[A^*]_0[B]_0 t. \tag{1.121}$$

Now we can substitute this result into Eq (1.120) to find the dynamics of $[C^*]$ at short times, again assuming that we start with plenty of $[C]$ and none of the activated version:

$$\frac{d[C^*]}{dt} \approx k(k[A^*]_0[B]_0 t)[C] = \{k^2[A^*]_0[B]_0[C]_0\}t \tag{1.122}$$

$$\Rightarrow [C^*] \approx \left(\frac{1}{2}k^2[A^*]_0[B]_0[C]_0\right) t^2 \tag{1.123}$$

So we see that the initial rise of $[B^*]$ is as the first power of time, the rise of $[C^*]$ is as the second power, and hopefully you can see that if the cascade

continued with C^* activating D, then $[D^*]$ would rise as the third power of time, and so on. In general, if we have a cascade with n steps, we expect that the output of the cascade will rise as t^n after we turn on the external stimulus.

Many people had the cascade model in mind for different biological processes long before we knew the identity of any of the molecular components. The idea that we could count the number of stages in the cascade by looking at how the output grows at short times is very elegant, and in Fig 1.11 we see a relatively modern implementation of this idea for the rod photoreceptors in the toad retina. It seems there really are three stages to the cascade!

This same basic idea of counting steps in a cascade has been used in very different situations. As an example, in Fig 1.12, we show the probability that someone is diagnosed with colon cancer as a function of their age. The idea is the same, that there is some cascade of events (mutations, presumably), and the power in the growth vs. time counts the number of stages. It's kind of interesting that if look only on a linear plot (on the left in Fig 1.12), you might think that there was something specifically bad that happens to people in their 50s that causes a dramatic increase in the rate at which they get cancer. In contrast, the fact that incidence just grows as a power of age suggest that there is nothing special about any particular age, just that as we get older there is more time for things to have accumulated, and there are several things that need to happen in order for cancer to take hold. It's quite amazing it is that these same mathematical ideas describe such different biological processes occurring on completely different time scales (years vs. seconds).

One can do a little more with the cascade model. If we think a little more (or maybe use the equations), we see that the maximum number of $[B^*]$ molecules that will get made depends on their lifetime $\tau = 1/k_-$: there is a competition between A^* activating $B \to B^*$, and the decay process $B^* \to B$. This same story happens at every stage, so again the peak number of molecules at the output will be proportional to some power of the lifetime of the activated molecules, and this power again counts the number of stages in the cascade, Thus the cell can adjust its sensitivity—the peak number of output molecules that each activated input A^* can produce—by modulating the lifetimes of the activated states. But if we change this lifetime, we also change the overall time scale of the response. Roughly speaking, the time required for the response to reach its peak is also proportional to τ. So we expect that if a cell adjusts its gain by changing lifetimes, then the gain and time to peak should be related to each other as gain $\propto t_{\rm peak}^n$, where there are n stages in the cascade; of course this value of n should agree with what

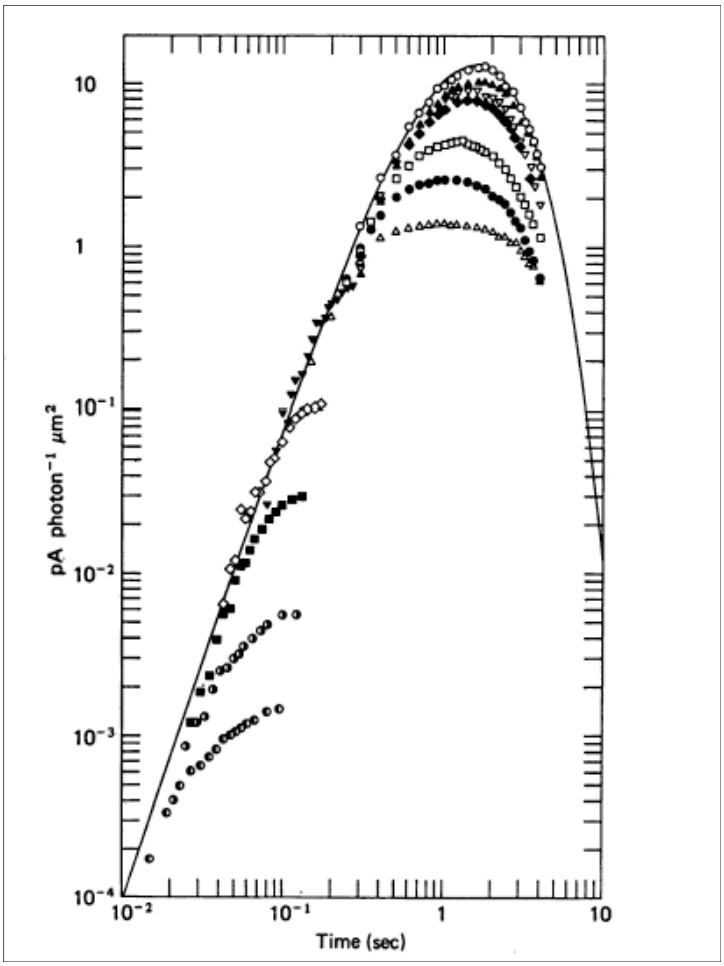

Figure 1.11: Kinetics of the rod photoreceptor response to flashes of light. The data points are obtained by measuring the current that flows across the cell membrane as a function of time after a brief flash of light. Different shape points correspond to brighter or dimmer flshes, and the response is normalize by taking the current (in pA, picoAmps; pico = 10^{-12}) and dividing by the light intensity (in photons per square micron). The lowest intensity flashes give the highest sensitivity, but it's hard to see the response at very early time because it's so small. As you go to brighter flashes you can see the behavior at small times, but then as time goes on the response tends to saturate so what is shown here is just the beginning. Solid line is $r(t) = A\exp(-t/\tau)[1-\exp(-t/\tau)]^3$, which starts out for small t as $r(t) \propto t^3$. From DA Baylor, TD Lamb & K–W Yau, The membrane current of single rod outer segments. *J Physiol* **288,** 589–611 (1979).

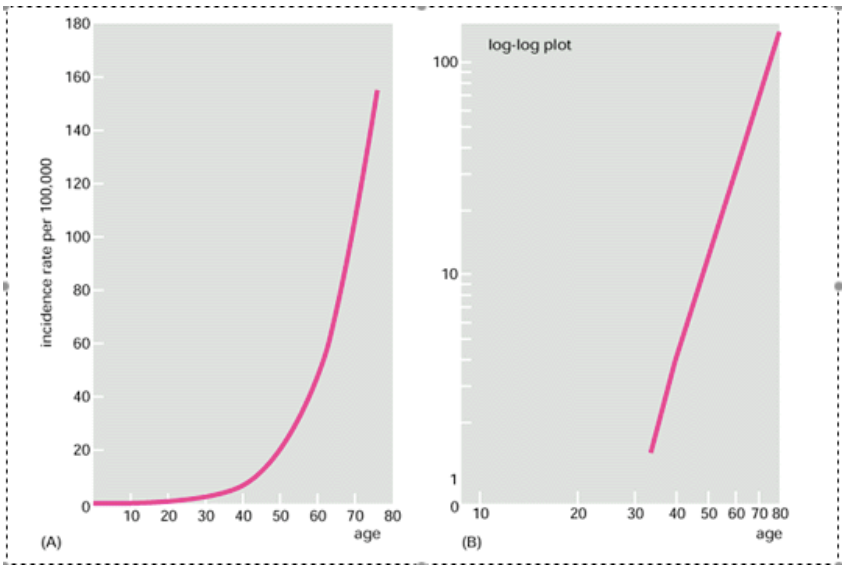

Figure 1.12: Incidence of colon cancer as a function of age. The original data, collected by C Muir et al (1987), refer to women in England and Wales, and are expressed as the number of diagnoses in one year, normalized by the size of the population. At left the data are plotted vs age on a linear scale, and on the right the are replotted on a log–log scale, as in Fig 1.11. What we show here is reproduced from *Molecular Biology of the Cell, 4th Edition*, B Alberts et al (1994). In the next version of these notes we'll go back and look at the original data.

we find by look at the initial rise in the output vs. time. A series of lovely experiments in the 1970s showed that this actually works!

What's nice about this example is that people were using it to think about how your retina adapts to background light intensity long before we had the slightest idea what was really going inside the cells. The fact that simple models could fit the shape of the response, and that these models suggested a simple view of adaptation, was enough to get everyone thinking in the right direction, even if none of the details were quite right the first time through. This is a wonderful reminder of how we should take seriously the predictions of simple models, and how we can be guided to the right picture even by theories that gloss over many details. Importantly, this works just as well inside cells as it does for more traditional physics problems.