Biochemistry 323
Systems Biology

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1 Introduction

Mathematical models are important tools for biologists to address specific research questions. In this section of the course we will look at how to construct a few example models and how to analyze and interpret the results we obtain from calculations and computer simulations.

The specific topics we will be addressing are:

- HIV/AIDS and treatment
- Blood glucose homeostasis
- Bacterial growth
- Kinetics and energetics of chemical reactions
- Membrane transport
- Enzyme catalyzed reactions
- Networks of reactions
- Metabolic regulation

Models can be very useful tools, not only to communicate, but also in the sciences where we often use models as simplified representations of our research systems.

What is a model? A model is a description/interpretation/representation of a system, where a system can be any interconnected set of components. These components are often treated as units of which the internal structure is ignored.

How would you decide whether a model is good or not? Models are tools and their quality should be judged by how good they fulfil their function.

Scientific models enable us to:

- *Understand* the system that is being modeled (give a quantitative description of the system on the basis of the characteristics of the components)
- Make *predictions* of future states (or otherwise unknown states)
- *Control* the system to produce a certain output (by manipulation of parameters on the basis of model simulations)

Classification of models

What types of models do we distinguish?

- descriptive (verbal)
- graphical (metabolic schemes)
- 3-D physical (stick and ball models)
- mathematical (e.g. algebraic or differential equations)

How can we further classify mathematical models?

- mechanistic versus phenomenological
- dynamic versus static (i.e. has an explicit representation of future system states)
- continuous time versus discrete time models
- spatially heterogeneous or homogeneous
- stochastic versus deterministic (parameters undergo random changes or are constant)

In this course we will focus on mathematical models using ordinary differential equations. These models can be classified as: deterministic, dynamic, continuous time, spatially homogeneous and mostly mechanistic.

Metabolic regulation

One of the applications of models in systems biology is that they can help us to gain a better understanding of metabolic regulation. To do this, we contrast the classical view of metabolic regulation (as it is presented in textbooks) with the functional view of metabolism. You will see that there are great shortcomings in the way in which textbooks present these concepts. We will then explore various new concepts and tools for analysing and describing such systems of coupled reactions:

- rate characteristics
- kinetics of chemical and enzymatic reactions
- thermodynamics, Gibbs free energy and the driving force for a chemical reaction
- metabolic control analysis

These tools will then be applied to the study of a few real-life metabolic pathways. In particular, we will show the flux through these pathways to be controlled by the demand for their end-products, i.e., the control lies outside of what has traditionally been considered the "pathway". This notion which is in stark contrast to the "rate-limiting step" of the classical view. But enough of this introduction, let's get going . . .

2 The spread of disease in a population: HIV

Mathematical models have helped scientists to understand otherwise hidden aspects of diseases allowing them to produce testable predictions about how diseases spread, and to generated forecasts that improve the efficacy of prevention and health care programs. The accuracy of these models is dependent on the parameter values which are often difficult to determine, but it is relatively easy to run the models with a range of parameter values and estimate the probability of different scenarios.

In this section we will use a mathematical model that was developed to describe the effect of antiretroviral therapy on the spread of HIV. The model presented here is a simplified version of the model described in Blower et al., 2000, Science, 287: 650-655.

2.1 HIV/AIDS and ART

In the early 1980's the first cases of the disease that has become known as AIDS (acquired immunodeficiency syndrome) were diagnosed in the United States. Since then the disease has reached epidemic proportions having caused more than 20 million deaths worldwide (Joint United Nations Programme on HIV/AIDS 2004b).

Infection with the human immunodeficiency virus (HIV) leads to a collapse of the immune system (AIDS) and a susceptibility to diseases such as Karposi's sarcoma, pneumocystis and tuberculosis. HIV is transmitted via exchange of bodily fluids. Once inside the body, HIV infects white blood cells by attaching to the CD4 protein of helper T cells, macrophages, and dendritic cells. The genetic material of HIV is first reverse transcribed into DNA and subsequently incorporated into the hosts genetic material, where it may remain latent or becomes activated. In the latter case HIV can produce hundreds of daughter viruses per day per host cell. These viruses go on to infect other CD4 bearing cells, repeating the process and destroying helper T cells which eliminates the very cells that recognise and fight other infections.

Early on in the epidemic, the median period between infection with HIV and the onset of AIDS was about ten years, while the median survival time following the onset of an AIDS-associated condition was just under one year. Sine then, survival statistics have improved dramatically with the development of effective antiretroviral therapies. These involve various drug combinations that have allowed people to live longer with HIV.

2.2 Why a mathematical model?

Blower et al. (2000) constructed a mathematical model to predict how drug therapy might affect the number of new cases of HIV and the number of deaths due to AIDS. In using their model to predict the future course of HIV, the authors pointed out that uncertainty exists in most model parameters, such as the effect of ART on survival and infectivity, as well as changes in the rate of risky behaviour. The authors thus allowed the parameters to be drawn from a range of plausible values rather than assigning one specific number to each. They then drew each parameter from its range and used 1000 different combinations of parameters to predict the spread of HIV.

2.3 The model

This model consists of three subpopulations (variables): uninfected individuals, $X(t)$, infected individuals on treatment, $Y_T(t)$, and untreated infected individuals, $Y_U(t)$.

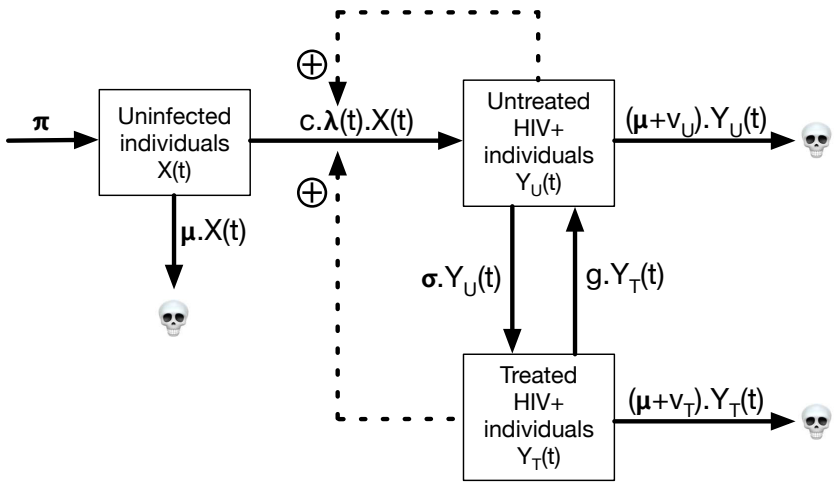

Figure 1: The Blower model of HIV infection and ART. Solid arrows indicate transfer of individuals between groups. Dashed arrows indicate effects that a group has on a specific process (with a + implying an acceleration). Mathematical expressions on arrows indicate the rates of processes.

The uninfected population is affected by three processes: 1) birth at a rate π per year; 2) natural death at a rate proportional to μ per uninfected individual per year and 3) new infections due to behaviour that puts them at risk of contracting HIV. This last process is proportional to the number of contacts one person has per year c, and the probability that a partner is HIV+ times the probability of acquiring HIV from this individual (a.k.a. the per capita force of infection) $\lambda(t)$.

The overall change in the population of uninfected individuals over time, is described by the equation

$$\frac{d\,X(t)}{dt} = \overbrace{\pi}^{\text{birth}} - \overbrace{\mu \cdot X(t)}^{\text{natural death}} - \overbrace{c \cdot \lambda(t) \cdot X(t)}^{\text{new infections}} \tag{1}$$

Here

$$\lambda(t) = \frac{\beta_U \cdot Y_U(t) + \beta_T \cdot Y_T(t)}{N(t)}$$

Specifically, if $N(t)$ is the total number of individuals, $N(t) = X(t) + Y_U(t) + Y_T(t)$, then the probability that an individual is HIV+ but not undergoing treatment is $Y_U(t)/N(t)$; such individuals tend to have a higher infectivity β_U. Similarly, the probability that an individual is HIV+ and undergoing treatment is $Y_T(t)/N(t)$; such individuals tend to have a lower infectivity, β_T.

Untreated individuals arise from 1) new infections and 2) treated individuals who abandon treatment at a rate proportional to g per person per year. They die due to 1) natural causes at a rate proportional to μ and 2) AIDS at a rate proportional to v_U per person per year, and they take up treatment at a rate proportional to σ per person per year:

$$\frac{d\,Y_U(t)}{dt} = \overbrace{c \cdot \lambda(t) \cdot X(t)}^{\text{new infections}} + \overbrace{g \cdot Y_T(t)}^{\text{abandon treatment}} - \overbrace{\mu \cdot Y_U(t)}^{\text{natural death}} - \overbrace{v_U \cdot Y_U(t)}^{\text{AIDS death}} - \overbrace{\sigma \cdot Y_U(t)}^{\text{take treatment}} \tag{2}$$

Treated individuals arise from untreated individuals who take up treatment at a rate proportional to σ per person per year. They die due to 1) natural causes at a rate proportional to μ and 2) AIDS at

a rate proportional to v_T per person per year, and they abandon treatment at a rate proportional to g per person per year:

$$\frac{d\,Y_T(t)}{dt} = \overbrace{\sigma \cdot Y_U(t)}^{\text{take treatment}} - \overbrace{g \cdot Y_T(t)}^{\text{abandon treatment}} - \overbrace{\mu \cdot Y_T(t)}^{\text{natural death}} - \overbrace{v_T \cdot Y_T(t)}^{\text{AIDS death}} \tag{3}$$

Equations 1, 2 and 3 are the ordinary differential equations (ODEs) of the model and the symbols π, μ, c, β_U, β_T, g, v_U, v_T, and σ are its parameters. These equations are solved using software with numerical differential equation solver functionality such as Wolfram Mathematica. The solution will be the time-dependent functions for the three variable $X(t)$, $Y_U(t)$, and $Y_T(t)$ that describe how the three subpopulations change over time. To obtain such a solution, initial (time = 0) values for the variables and parameter values are required. Fig. 2 displays an example of such a solution (also called a simulation result). These kinds of simulation results allows one to address questions of treatment efficacy and long term impact.

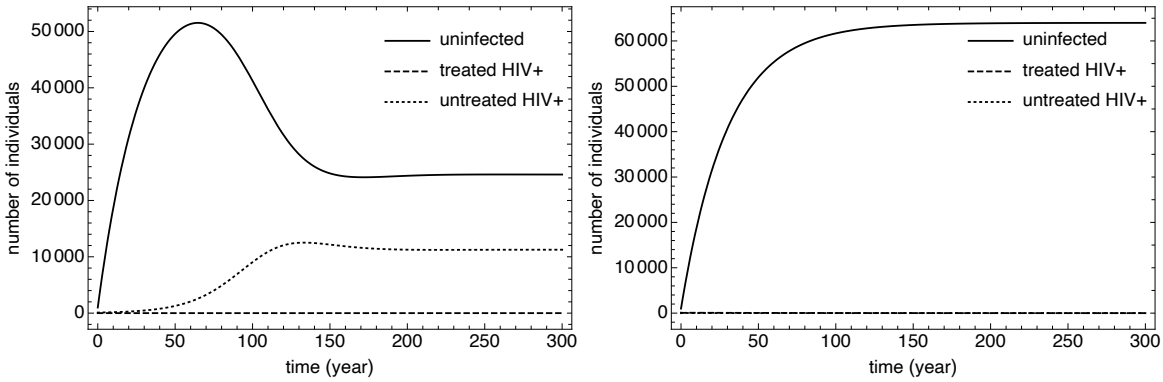

Figure 2: Two simulation results for the Blower model of HIV infection and ART. These results were obtained for the same paramater set, apart from σ which was 0 for the left panel (NO treatment), and non-zero for the right panel (with treatment).

3 Blood glucose homeostasis

In a previous Biochemistry course on metabolism you may have learnt that the glucose-insulin hormonal system is important for the maintenance of a constant glucose level in the blood (see Fig. 3).

3.1 The physiology of glucose homeostasis

Cells require a continuous input of free energy, usually supplied in the form of glucose, transported via the bloodstream. The continuous supply is maintained via a homeostatic control of the bloodstream glucose concentration, close to 5.5 mM. This homeostatic control is dependent on the hormonal system of insulin and glucagon.

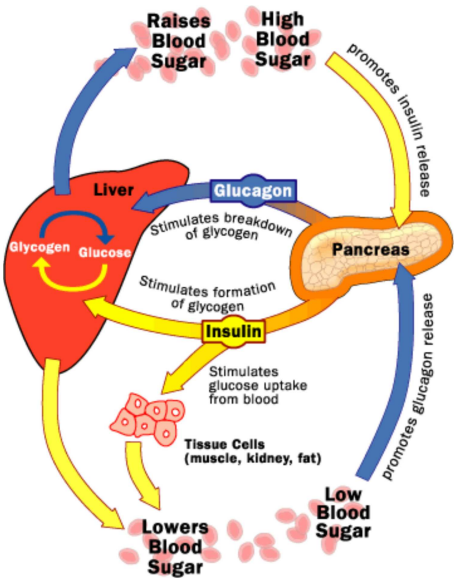

Figure 3: Blood glucose homeostasis mediated by insulin and glucagon.

Glucose enters the bloodstream during digestion of a meal leading to an increase in the glucose concentration. Upon sensing this increase in blood glucose concentration the Beta-cells in the islets of Langerhans in the pancreas secrete insulin. Insulin stimulates glucose uptake, especially by adipose and muscle cells, and stimulates the liver to convert glucose to glycogen.

Upon a lowering of the glucose concentration below the basal level another hormone is secreted from the pancreas, glucagon. This hormone stimulates the conversion of glycogen back to glucose.

A failure in the normal glucose-insulin regulation leads to a number of diseases: 1) Type I diabetes, due to low excretion of insulin, 2) Type II diabetes, due to low concentrations of insulin receptors. Both Type I and II forms of diabetes lead to high blood glucose concentrations, leading to low blood pH levels. In contrast to these diseases with a lower (effective) insulin concentration, in obese individuals a high rate of insulin production is observed.

3.2 Why a mathematical model?

Mathematical models of the glucose-insulin system have been important for 1) increasing our understanding of the (dys)-regulation of the bloodstream glucose concentration and importantly 2) as a

diagnostic tool. For the latter functionality a person is subjected to a well-controlled medical diagnostic procedure, e.g. intravenous injection of a known concentration of glucose, and the bloodstream glucose and insulin concentrations are measured in a so-called FSIGT-test (frequently-sampled intravenous glucose tolerance test - see Fig. 4). This data can be used to obtain a number of metabolic indices, e.g. insulin sensitivity, glucose effectiveness, first and second phase pancreatic responsivity.

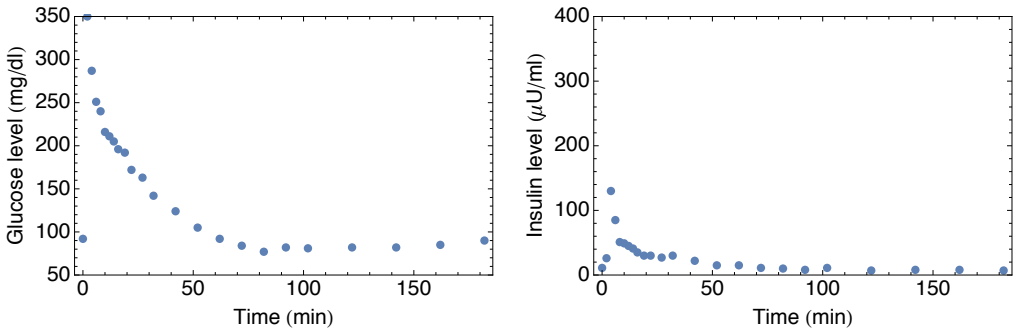

Figure 4: Clinical data set of glucose pulse and insulin concentrations. One international unit of insulin (1 U) is defined as the "biological equivalent" of 34.7 μg pure crystalline insulin.

3.3 The model

Since its development, the model (Bergman et al., 1979, Am. J. Physiol., 236, E667–77) has been extended to include long term glucose effects, including Beta-cell growth and can also successfully model different diabetic disorders. For the purpose of this course, we will focus on short term glucose effects and the minimal glucose model is sufficient.

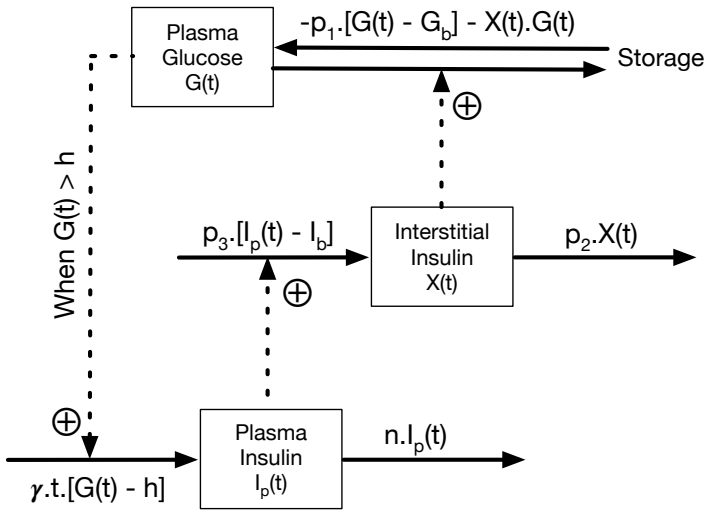

Figure 5: Schema of the Bergman model for blood glucose homeostasis. Solid arrows indicate transfer of species. Dashed arrows indicate effects that a species has on a specific process (with a + implying an acceleration). Mathematical expressions on arrows indicate the rates of processes.

The model (see Fig. 5) has three variables: the plasma glucose concentration $G(t)$, the plasma insulin concentration $I_p(t)$, and the interstitial tissue insulin concentration $X(t)$. Glucose is degraded

via two mechanisms, the first is dependent on the difference between the blood and basal glucose concentrations (insulin independent) and the second dependent on insulin:

$$\frac{d\,G(t)}{dt} = -\overbrace{p_1 \cdot [G(t) - G_b]}^{\text{insulin independent}} - \overbrace{X(t) \cdot G(t)}^{\text{insulin dependent}} \quad . \tag{4}$$

The plasma insulin concentration production is proportional to the product of the time period (after glucose addition) and the glucose concentration above a certain threshold h, while its degradation is proportional to its concentration:

$$\frac{d\,I_p(t)}{dt} = \overbrace{\gamma \cdot [G(t) - h] \cdot t}^{\text{production}} - \overbrace{n \cdot I_p(t)}^{\text{degradation}} \tag{5}$$

The interstitial insulin formation is dependent on the difference between the plasma and basal insulin concentrations I_b, while its degradation is linear with its own concentration:

$$\frac{d\,X(t)}{dt} = \overbrace{p_3 \cdot \left[(I_p(t) - I_b\right]}^{\text{formation}} - \overbrace{p_2 \cdot X(t)}^{\text{degradation}} \,. \tag{6}$$

The overall changes in the variable (metabolite) concentrations over time, are described by these three differential equations.

Here p_1, p_2, p_3, γ and n are model parameters of which the values are determined by fitting the model simulation results to experimental data. This is done by scanning over a range of possible values for the parameters and identifying the combination of values that brings the simulation results as close as possible to the data points (using e.g. a so-called sum of squared differences approach). Fig. 6 shows a typical result of the model simulation (lines) in addition to the clinical FSIGT data (dots) after such a fitting procedure.

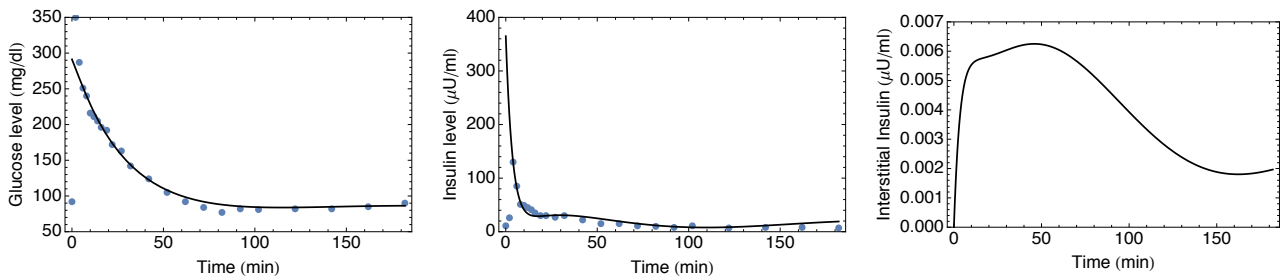

Figure 6: Clinical data (dots) and fitted model simulation results (lines) of glucose and insulin levels after feeding.

In practice the model parameters (obtained by fitting the model equations to clinical FSIGT data) are used to calculate metabolic indices. These are typically insulin sensitivity (Si) defined as the ratio of the model parameters p_3/p_2, which should be between 2.1 and 18.2×10^{-4} ml/min/μU insulin, and glucose effectiveness (Sg) equal to p_1 which should be between 0.0026 and 0.039 min^{-1} for healthy individuals.

4 Bacterial growth

The capacity of microorganisms to grow is often the sole criterion used to assess whether an organism is alive. An organism is usually considered dead when it fails to grow upon prolonged incubation in a growth supportive medium. Although we cannot see individual microorganisms, after a number of cell divisions we can observe groups of them as colonies on solid media or as turbidity in liquid media. To measure growth of microorganisms, increases in time of either cell number or cell mass are used.

Bacteria reproduce via binary fission, a process in which, so far as is known, the contents of the mother cell is distributed equally over the two daughter cells. In this process the mother cell disappears as the two daughter cells are created. Binary fission should lead to identical daughter cells with identical physiological properties. In theory such daughter cells should divide synchronously but this rarely happens and a bacterial culture consists of a population of cells with a distribution of growth rates and cell sizes. We usually refer to the mean values of such distributions to describe the culture. The newly formed daughter cells take up nutrients and increase in biomass and size until they also divide. For growth to be balanced a cell must synthesize a range of macromolecules in a coordinated way. The building blocks for these macromolecules can be derived from a relatively small number of elements, present in all living cells: carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, magnesium, potassium and a number of so-called trace elements. All of these elements must be present in a utilizable form along with water in order for growth to be possible. Free energy for the growth process is often derived from oxidation of sugars but other mechanisms (such as photosynthesis) exist.

4.1 The rate of growth

In a constant environment a population of cells will obtain a constant generation time, i.e. the time it takes for a number of organisms (N) to grow to a population of $2N$, also called the generation time or doubling time, t_d (hours), is constant. The growth process can be represented as:

$$N_0 \rightarrow 2N_0 \rightarrow 4N_0 \rightarrow 8N_0 \rightarrow 16N_0 \rightarrow 32N_0$$

or:

$$2^0N_0 \rightarrow 2^1N_0 \rightarrow 2^2N_0 \rightarrow 2^3N_0 \rightarrow 2^4N_0 \rightarrow 2^5N_0 \rightarrow 2^nN_0$$

Where n represents the number of doubling that have occurred after a certain time interval t ($n = \frac{t}{t_d}$). Now the number of cells present in a culture at any time point t, N_t, can be expressed as a function of the number of cells present at t=0, N_0 (typically an inoculation size) and the doubling time:

$$N_t = N_0 \cdot 2^{\frac{t}{t_d}} \quad \text{or} \quad \frac{N_t}{N_0} = 2^{\frac{t}{t_d}}. \tag{7}$$

Taking natural logarithms we obtain

$$\begin{aligned} \ln(N_t) &= \ln\left(N_0 \cdot 2^{\frac{t}{t_d}}\right) = \ln(N_0) + \ln\left(2^{\frac{t}{t_d}}\right) \\ &= \ln(N_0) + t \cdot \frac{\ln(2)}{t_d} \end{aligned} \tag{8}$$

Thus, if t_d is indeed constant (note that this is the only criterium), plotting the natural logarithm of the cell number ($\ln(N_t)$) against time should result in a straight line with a slope numerically equal to $\frac{\ln(2)}{t_d} = \frac{0.693}{t_d}$.

Taking the derivative with respect to time on both sides of eq. 8 yields:

$$\begin{aligned} \frac{d\ln(N_t)}{dt} &= \frac{d}{dt}\left(\ln(N_0) + t \cdot \frac{\ln(2)}{t_d}\right) \\ \frac{d\ln(N_t)}{dN_t} \cdot \frac{dN_t}{dt} &= 0 + \frac{\ln(2)}{t_d} \\ \frac{1}{N_t} \cdot \frac{dN_t}{dt} &= \frac{\ln(2)}{t_d} = \mu. \end{aligned} \tag{9}$$

The fraction $\frac{1}{N_t} \cdot \frac{d(N_t)}{dt}$ is called the specific growth rate and is given the symbol μ (unit: time^{-1}). Often it is better to use μ than to use growth rate (i.e. $\frac{d(N_t)}{dt}$) since the latter is dependent on the actual cell number while μ is expressed per unit of cell number.

Experimentally it is often more convenient to measure biomass than to measure the number of cells. Biomass can be determined as dry weight, using an analytic balance after drying a precisely measured volume of culture. To determine the cell number, sophisticated equipment such as a Coulter counter (or elaborate methods such as plate counting) must be used. The method that is used most often to get a measure for biomass is to determine the absorbance of a bacterial culture (optical density) using a spectrophotometer (wavelengths between 450 and 600 nm are used).

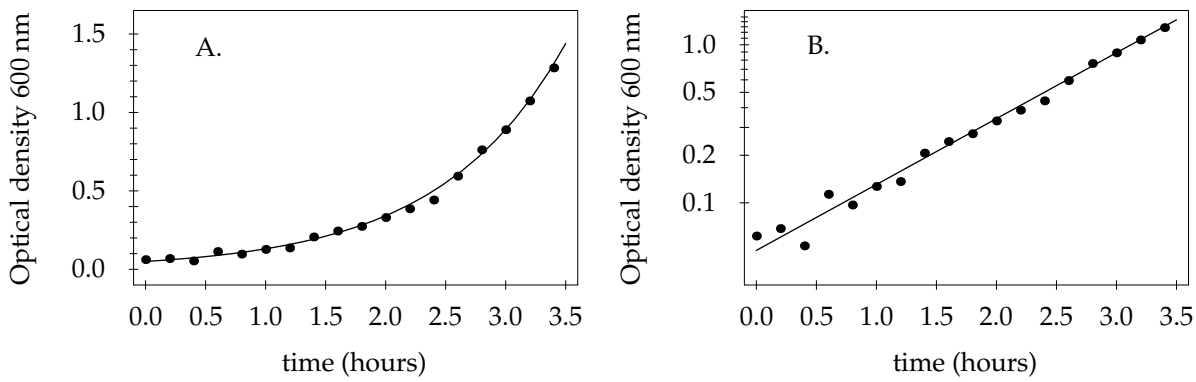

Figure 7: *Exponential growth.* A. Plot of optical density at 600 nm against time, linear axes B. Semi logarithmic plot of optical density against time.

At first it might seem surprising that one can use measurements of both biomass and cell number to determine the specific growth rate. In a synchronously dividing cell culture the biomass concentration would increase exponentially but cell number would have a stepwise increase (a doubling in cell number after each doubling time). However synchronously dividing cell cultures are very rare and usually a cell culture will show a distribution of cell sizes thereby eliminating the stepwise increase in cell number. With x as the culture biomass concentration (g dry weight$\cdot$ L^{-1}, or less conventionally mM), the specific growth rate can be expressed as:

$$\mu = \frac{1}{x} \cdot \frac{dx}{dt} \tag{10}$$

Independent of whether biomass, optical density or cell number is measured the unit of specific growth rate is time^{-1}. One can choose whatever units of time is suitable but it must be specified.

When one is interested to calculate the biomass concentration or the cell number at a time point t it is often useful to use the following formula, which can be derived by substituting (9) into (8):

$$\ln(\frac{N_t}{N_0}) = \mu \cdot t \tag{11}$$

and taking the exponent:

$$N_t = N_0 \cdot e^{\mu \cdot t} \tag{12}$$

Microorganisms have an enormous growth potential which can be easily demonstrated in the following example. *Escherichia coli*, a common gut bacterium is capable to divide every 20 minutes when grown under favourable conditions. Thus, starting with 1 *E. coli* cell after approximately 9.75 hours of growth a cell number equal to the human population is reached ($\pm$ 7 billion). With a bacterial weight of 9.5×10^{-13} g/cell this amounts to about 5 milligram of bacteria. However if we would let the culture grow at this rate for 3 days then the bacterial mass would equal the mass of the earth 5.98×10^{24} kg! Clearly, this will not happen in reality. The reason that the growth rate decreases over time is that the bacteria change their environment, ultimately leading to the cessation of growth. First, growth can lead to the accumulation of toxic products, where one should also think of protons and hydoxyl ions, leading to changes in the culture pH. Second, during growth the bacteria consume nutrients and these might become limiting, thereby impeding growth.

4.2 Monod description of growth

The only prerequisite for exponential growth is a constant specific growth rate as can be seen from eq. 12. Thus far we have discussed in qualitative terms what possible factors could influence the specific growth rate, such as a change in pH, product accumulation or a decrease in substrate concentration. An often used quantitative description of growth rate is the Monod equation:

$$\mu = \frac{\mu_m \cdot s}{K_S + s} \tag{13}$$

with μ_m (hours^{-1}) the growth rate observed at saturating substrate concentrations and K_S (mM) the substrate concentration at which half maximal growth rate is observed. The equation is identical to the Michaelis Menten equation for enzyme kinetics with the V_M and K_M being replaced by μ_m and K_S respectively. It is rather surprising that the growth rate of a complex machinery such as a living cell with thousands of enzymes can be modelled with the same equation as used for a single enzyme. Not only does it appear as if the cell can be modelled as a single enzyme, the activity of this enzyme appears to be dependent on a single substrate only. Clearly the description is very simple and does not take the afore mentioned effects of pH and product accumulation into account. However such effects can be incorporated into the Monod description which has been remarkably successful and is widely used. It should however be noted that no mechanistic interpretation for the Monod description of growth exists and its success is solely based on the equation being able to give good fits to experimental data. The kinetic parameters used in the Monod equation, μ_m and K_S are usually obtained by fitting to experimental data points of specific growth rate at different substrate concentrations. A linear fit can be made after a transformation of the data points, i.e. using a Lineweaver-Burk plot, but a direct non-linear fit to the data points is more accurate.

4.3 The batch growth cycle

Although the use of batch cultures for physiological studies is limited, batch cultures are very popular to obtain cells in a quick and easy way. Certainly for molecular studies where the history of the cells is often not very important, batch cultures are widely used. The best known form of batch culture is the Erlenmeyer flask (see Fig. 8), which can be used for aerobic culturing of microorganisms if well agitated and when care is taken that the biomass concentration is not too high.

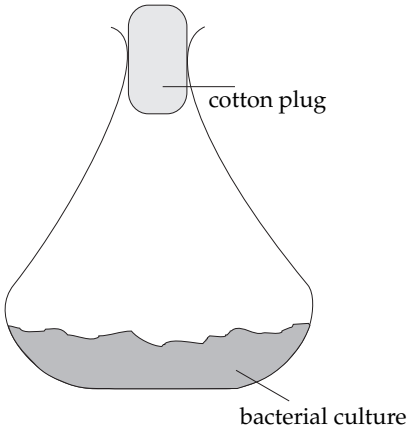

Figure 8: Erlenmeyer flask as a typical example of a batch culture.

If we take a look at a real growth curve (Fig. 9) we can distinguish three (sometimes four) growth phases: the *lag*, *log* and *stationary* phase. The log phase during which the cells grow exponentially is the phase we have been discussing thus far. In the lag phase bacteria grow slower than in the log phase which is due to metabolic adaptations of the bacteria to the medium. In the stationary phase growth rate is also slower than in the log phase and eventually ceases completely but here this is due to either toxic products that are formed during growth or due to nutrient limitation. Dependent on how long the culture is followed, a starvation phase can be observed in which the cells die off and optical density decreases.

The growth cycle as observed in Fig. 9 reflects some of the capacity of microorganisms to adapt themselves to changes in the environment. One should realize that this so-called growth cycle of the culture is more a characteristic of the closed system than of the bacterium. With respect to their adaptability, microorganisms are much more versatile than, for instance, human cell lines which can only survive in a narrow range of conditions. One should look at this difference between cells from unicellular and multicellular organisms in relation to the environment that surrounds them. Conditions in the human body are kept relatively constant and cells will not experience any great changes in their growth conditions. Bacterial cells on the other hand are very limited in their ability to control their environment and have acquired control mechanisms to change themselves instead. Bacteria are able to adapt themselves to such an extent that it is nonsense to speak about a *normal* bacterial cell. When referring to the physiological or structural state of a bacterial cell it is essential to give the precise growth conditions of that bacterial cell.

Most experiments are performed in the exponential growth phase unless a researcher is specifically interested in the lag or stationary phase. The log phase is considered to be the best defined, if it were only for the constant growth rate of the bacteria. A method that is sometimes used to keep bacteria for a long period in the log phase is by diluting an exponentially growing culture in fresh

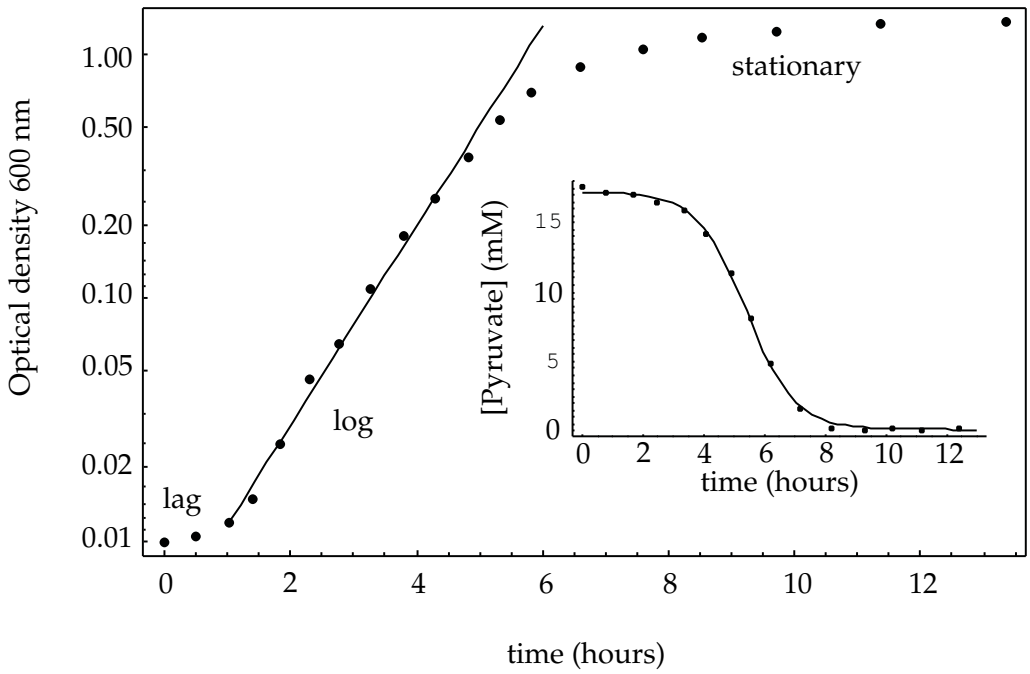

Figure 9: *Growth cycle of a batch culture.* The different phases of the growth cycle, *lag*, *log* and *stationairy* phase are shown for a batch culture of *Enterococcus faecalis* with pyruvate as the primary free energy source. Optical density at 600 nm is plotted on a semi-logarithmic scale against time. The insert shows the decline in pyruvate concentration during the experiment.

medium. Thereby the accumulation of toxic products is prevented and all nutrients will be present in surplus. Since the bacteria are diluted in the same media as in which they were growing, there will generally not be a lag phase upon such a dilution. Using this dilution method bacteria can be kept in the exponential phase for a long time and eventually a condition called balanced growth can be reached. This is a situation in which not only the specific growth rate is constant (the sole necessary condition for exponential growth) but also the average concentration of all intracellular metabolites and the average cell size of the population. Balanced growth is as close to a steady state as one can get in a closed system. Typical examples of closed systems that are routinely used in laboratories are Erlenmeyer flasks, such cultures are also often referred to as batch cultures as they are used for growing a batch of microorganisms.

4.4 Growth yield

Although a single bacterial cell cannot control the environment, as a population they can affect it, ultimately changing the environment to such an extent that growth is no longer possible. Before we stated that changes in the pH of the culture due to product formation (or substrate consumption) is one of the factors slowing down growth rate in dense cultures. This effect can be overcome by automatically adjusting the pH value, and doing so usually leads to a higher final population density but the increase might be small. With aerobic organisms the availability of oxygen is often a limiting factor in cultures of high densities. This might seem surprising since oxygen is readily available in the atmosphere, but here we should realize that the solubility of oxygen (and the dissolved oxygen is what counts for the bacteria) is low (5-7 μg/ml at 20 °C). Thus in a culture with a

high oxygen consumption rate, the rate at which oxygen dissolves into the culture becomes limiting for growth. Even under *an*aerobic conditions with automatic pH adjustment bacterial growth will ultimately slow down and usually no more than a few milligrams of bacteria per milliliter will be obtained. That this must occur can be understood on basis of the nutrient consumption by the bacteria. As can be seen in Fig. 9 growth stops when the substrate pyruvate (insert of Fig. 9) is consumed. More importantly it can be seen from the figure that the pyruvate consumption increases with time, i.e. with increasing culture density. Generally a proportionality between biomass synthesis and substrate consumption rate is observed when cells are incubated under constant conditions. That is:

$$\frac{1}{x} \cdot \frac{dx}{dt} \propto -\frac{1}{x} \cdot \frac{ds}{dt} \quad \text{or} \quad \mu = Y_s \cdot (-q_s) \tag{14}$$

with q_s the specific substrate consumption rate defined as $\frac{1}{x}\frac{ds}{dt}$ with units (mmol $\cdot$ g^{-1}biomass $\cdot$ h^{-1}) and $Y_s = \frac{dx}{-ds}$ the yield value (g biomass $\cdot$ g^{-1} substrate, or when expressed as the molar yield value g biomass $\cdot$ mol^{-1} substrate), i.e. the amount of biomass formed per amount of substrate consumed. Note that q_s is expressed as change in substrate concentration and will thus be negative (i.e. the substrate is consumed). Strictly, the consumption rate would then be positive, however, we have decided to keep the negative value for the q_s. The molar yield value for glucose found with *E. coli* when grown aerobically on a simple salts medium is 90 (g organisms produced per mol of glucose consumed). With this knowledge we can calculate how long it takes for our rapidly growing *E. coli* in our previous example to finish the glucose in a 5 mg/ml glucose solution (27.8 mM) if we inoculate with 0.001 mg/ml of organisms. Using the Y_{glc} of 90 g/mol the 5 mg/ml will yield 2.5 mg/ml of *E. coli*. Assuming no lag phase and a doubling time of 20 minutes (i.e. specific growth rate is 2.08 h^{-1}) then after (using eq. 11) $\ln(\frac{2.5}{0.001})/2.08 = 3.76$ hours the bacteria would have consumed all glucose. Clearly, when incubated overnight such a culture would have entered stationary phase long before the cells could be harvested (even with a lag phase of several hours).

4.5 Substrate consumption and product formation rates

In experimental studies normally concentrations of metabolites and biomass are measured in the bacterial cultures. An example of such experimental values is shown in Fig. 9. Plotting the concentration changes against time will give us an indication how rapidly the substrate is consumed (or product and biomass is formed). Generally such rates can be expressed in two ways, firstly as volumetric production (or consumption) rates and secondly as specific rates (q). The volumetric rate (mM $\cdot$ h^{-1}) is the change in concentration per time while the specific rate (mmol $\cdot$ h $^{-1}$ $\cdot$ g^{-1} biomass) is normalized per biomass concentration. In a production process one might be interested in the total amount of a product formed in a specific process per unit time, irrespective of the biomass present. Then one would use the volumetric rate, by multiplying the volumetric rate with the volume of the culture one obtains the total amount of product formed per unit time. When the interest is more towards the organism, one would work with specific production rates. For instance when one compares two different strains with respect to their product formation rates it is important to compare the specific production rates which are independent of the biomass concentration.

In a batch culture the specific product formation rate (q_p) can be calculated from a series of product concentration (p), and biomass concentration (x) measurements. After plotting p against time, q_p at time t equals the slope of this plot at t divided by the biomass concentration at point t. In practice q_p is derived by first fitting curves through the data points of p and x, yielding the equations, p_t and

x_t respectively. Subsequently q_p is calculated as the time derivative of p_t divided by x_t:

$$q_p = \frac{dp_t}{dt} \cdot \frac{1}{x_t} \tag{15}$$

Data from a typical experiment is shown in Table 1.

time (hour)	glucose (mM)	ethanol (mM)	biomass (g dry weight)
5	49.74	0.447	0.006
6	49.61	0.681	0.009
7	49.41	1.017	0.013
8	49.13	1.501	0.018
9	48.73	2.195	0.026
10	48.16	3.193	0.037
11	47.33	4.624	0.054
12	46.15	6.674	0.078
13	44.46	9.607	0.112
14	42.04	13.79	0.160
15	38.62	19.72	0.229
16	33.81	28.06	0.325
17	27.14	39.61	0.458
18	18.26	55.00	0.636
19	7.746	73.22	0.846
20	0.760	85.33	0.986
21	0.016	86.62	1.000
22	0	86.65	1.001
23	0	86.65	1.001

Table 1: Experimental data of a typical batch culture.

Non-linear best fits were made on the data points of Table 1. The time derivative of the obtained equations was calculated and divided by the function fitted on the biomass data. The results (Fig. 10 give q_{glucose} (-18.2 mmol·g^{-1}dry weight · h^{-1}), q_{ethanol} (31.5 mmol·g^{-1}dry weight · h^{-1}) and μ (0.36 h^{-1}) in the initial phase of the experiment.

4.6 Extensive properties and mass balances

In building kinetic models it is important to first set up a set of balance equations for the extensive properties. Extensive properties such as the amount of biomass, substrate and product are additive over the system, i.e. the total amount of the property in the system is the sum of the property in the separate parts, or in other words, if you double the system all of the extensive properties will double. For instance the amount of substrate is an extensive property while the concentration of the substrate is not, also temperature and pH are not extensive properties. Balance equations are written for amounts of properties (i.e. concentrations are not conserved). Thus when dealing with the biomass concentration, x (mM or g dry weight· L^{-1}), we need to multiply the property with the

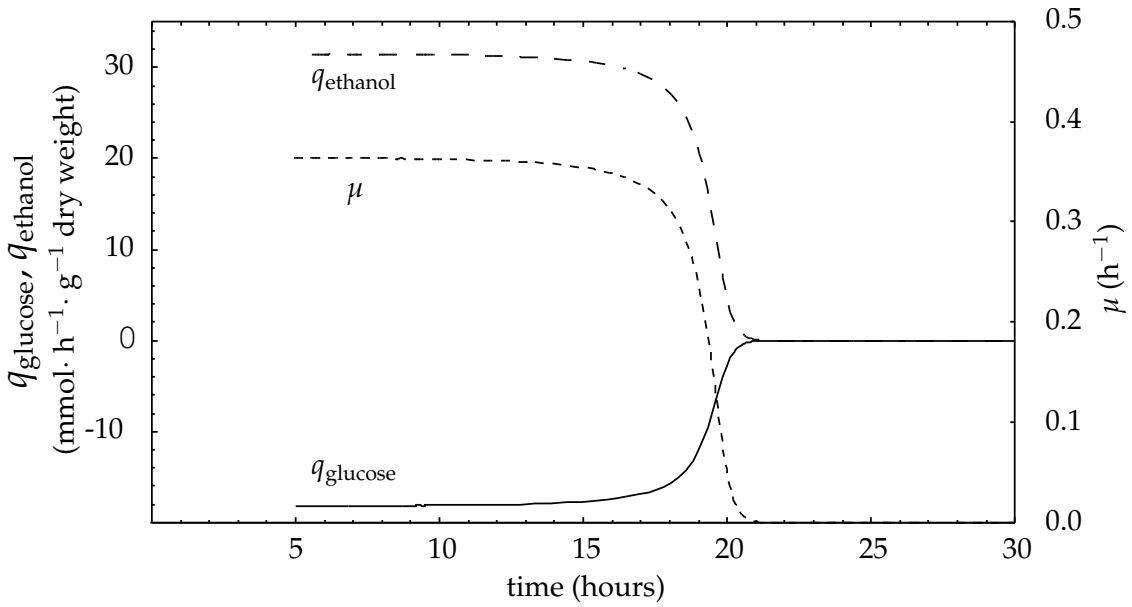

Figure 10: Specific rate of glucose consumption, ethanol production and specific growth rate in the modelled batch culture.

volume in which it resides to get the amount, i.e. $V \cdot x$ (moles or g dry weight). The accumulation of biomass would be,

$$\frac{d(V \cdot x)}{dt} \quad \text{or} \quad \frac{dV}{dt} \cdot x + \frac{dx}{dt} \cdot V \tag{16}$$

which simplifies to $\frac{dx}{dt} \cdot V$ when the volume is constant ($\frac{dV}{dt} = 0$) as in a typical batch culture. A general mass balance equation can be formulated as follows:

rate of accumulation	=	rate of transport	+	rate of production.
And for the specific case of biomass accumulation in the batch culture:				
$V \cdot \frac{dx_t}{dt}$	=	0	+	$\mu \cdot x_t \cdot V$

which is equal to eq. 10.

4.7 Model description of batch cultures

Let's now set out and try to make a mathematical description, a model, that captures some of the features of a batch culture. Our first model will be very simple. We assume yeast to be a catalytic unit that converts glucose into biomass and ethanol according to a fixed stoichiometry, i.e. as if it is a single chemical reaction. Growth rate is assumed to follow Monod kinetics and is only dependent on the glucose concentration. Experimentally a μ_m of 0.4 h^{-1}, a K_s of 5 mM and a biomass yield of 20 g/mol of glucose were determined. Assuming an elementary biomass composition of $C_4H_7O_2N$ with a "molecular weight" of 100, the molar yield is 0.2 mol of biomass/mol of glucose (in the second part of this course biomass composition is treated in more detail). Assuming that all glucose is either converted to biomass or ethanol the following reaction equation can be derived (focussing only on carbon ignoring all other elements):

$$C_6H_{12}O_6 \rightarrow 0.2 \cdot C_4H_7O_2N + 1.733 \cdot C_2H_5OH + 1.733 \cdot CO_2. \tag{17}$$

The following mass balance equations can be derived:

$$\begin{array}{rcccl} \text{rate of accumulation}(\text{mmol} \cdot \text{h}^{-1}) & = & \text{rate of transport} & + & \text{rate of production.} \\ V \cdot \frac{dx_t}{dt} & = & 0 & + & \mu \cdot x_t \cdot V \\ V \cdot \frac{ds_t}{dt} & = & 0 & + & -\frac{1}{Y_s} \cdot \frac{dx_t}{dt} \cdot V \\ V \cdot \frac{dp_t}{dt} & = & 0 & + & 1.733 \cdot \frac{1}{Y_s} \cdot \frac{dx_t}{dt} \cdot V \end{array}$$

Clearly these three differential equations are linearly dependent, reflecting that our system is actually a single reaction. Such a system contains only one state variable, if one of the variables s_t, x_t or p_t is known, the other two can be calculated. Choosing s_t as state variable, and s_0=50 mM, x_0=0.01 mM (0.001 g/L), $p[0]$=0, the following differential equation can be derived from the above balance equations:

$$\begin{aligned} \frac{ds_t}{dt} &= -\frac{1}{Y_s} \cdot \frac{dx_t}{dt} \quad (\text{mM} \cdot \text{h}^{-1}) \\ &= -\frac{1}{Y_s} \cdot (\mu) \cdot (x_t) \\ &= -\frac{1}{Y_s} \cdot (\frac{\mu_m s_t}{K_s + s_t}) \cdot (x_0 + (s_0 - s_t) \cdot Y_s) \\ &= -5 \cdot \frac{0.4 s_t}{5 + s_t} \cdot (10.01 - s_t \cdot 0.2) \end{aligned} \tag{18}$$

After solving the differential equation numerically on a computer, x_t and p_t can be calculated from s_t as follows:

$$x_t = x_0 + (s_0 - s_t) \cdot Y_s = 10.01 - s_t \cdot 0.2 \quad (\text{mM}) \tag{19}$$

$$p_t = (s_0 - s_t) \cdot Y_s^p = (50 - s_t) \cdot 1.733 \quad (\text{mM}) \tag{20}$$

Eqs 18 to 20 make up our model of the batch growth cycle. Note: to convert the awkward expression of biomass in (mM) into the commonly used (g/L) one should divide the above expression for x_t by 10. Plotting the result yields Fig. 11.

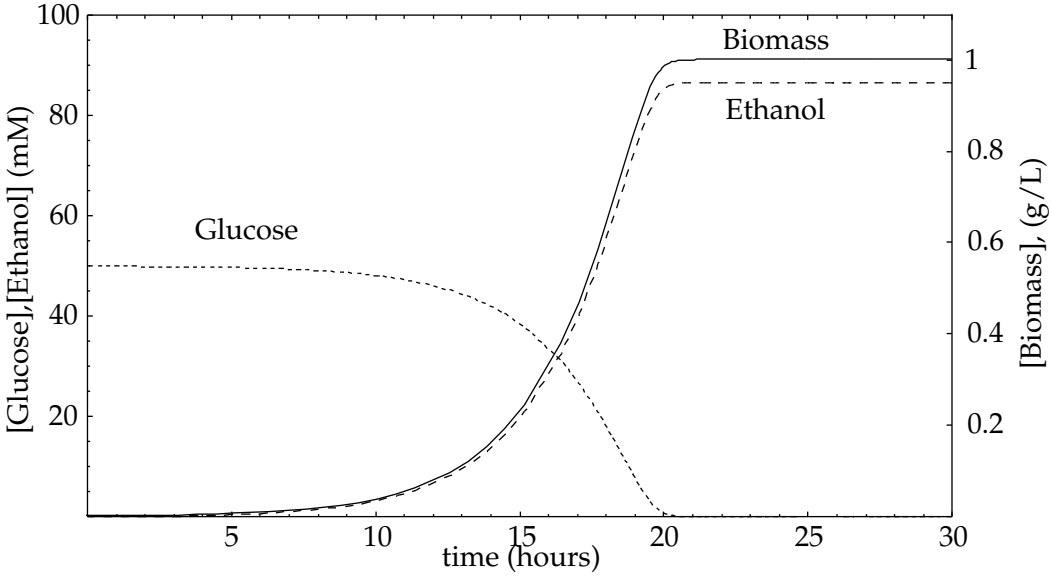

Figure 11: *Model description of the batch growth cycle.* Yeast growth on glucose was simulated using a simple model (eqs 18 to 20) and assuming Monod kinetics for growth (see text for details). The log phase ends when the glucose concentration becomes limiting for growth. Note the different axes for glucose and ethanol concentration (left y-axis) and biomass concentration (right y-axis), respectively.

5 The kinetics and energetics of chemical reactions

In the previous sections whole organs and bacterial cells were treated as single units and modelled as "black boxes" (i.e. input and output defined but no details of internal structure). However, as biochemists and molecular biologists, we want to understand what happens *inside* a living cell, because (i) we are scientists, and understanding and explaining is what science is all about, and (ii) we have this desire to manipulate things, which is not always a good thing, but which is nevertheless there. This understanding we seek is not any old understanding, but understanding at a deep level, the level of molecular reaction and interaction, i.e., a physico-chemical understanding.

5.1 Reversible and elementary reactions

At the fundamental level of chemical mechanism, the three basic building blocks for molecular events that occurs in living processes are:

1. an **association** between two molecules to form a non-covalently bound complex,

$$\mathrm{A} + \mathrm{B} \longrightarrow \mathrm{A} \cdot \mathrm{B} \tag{21}$$

2. a **dissociation** of a complex into two molecules,

$$\mathrm{A} \cdot \mathrm{B} \longrightarrow \mathrm{A} + \mathrm{B} \tag{22}$$

3. an interconversion where one molecule is chemically transformed into another (an **isomerisation**).

$$\mathrm{A} \longrightarrow \mathrm{B} \tag{23}$$

   This reaction could also depict the excitation of A by, for example, light.

These **elementary reactions** are defined to be **irreversible** (denoted by $\longrightarrow$). They never exist in isolation, but always in combination with each other. So, what we usually describe as a *chemical reaction* can always be broken down into a **mechanism** that consists of combinations of these three elementary processes. For example, the probable mechanism of the chemical reaction $\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{C}$ would be

$$\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{A} \cdot \mathrm{B} \rightleftharpoons \mathrm{C} \tag{24}$$

where C is A—B, the condensation product of A and B. Each half ($\rightharpoonup$ or $\leftharpoonup$) of the double arrow ($\rightleftharpoons$) denotes one of the elementary reactions. In principle, *all* chemical reactions are *reversible* and consist of a forward reaction and a reverse reaction.

5.2 Reaction rates—how fast?

A fundamental question of reaction chemistry is "how fast does an elementary process occur and what determines this rate?" All else follows from this question. So, let us ask it. Given the incredible diversity of the molecular world, the answer is astoundingly simple: the rate v of an elementary reaction is proportional ($\propto$) to the concentration of molecules involved, irrespective of the identity of the molecules. This fundamental law of chemistry is of course the famous *law of mass action*. For example, for the elementary reaction $\mathrm{A} + \mathrm{B} \longrightarrow \mathrm{A} \cdot \mathrm{B}$ this boils down to

$$v \propto a \qquad \text{and} \qquad v \propto b \tag{25}$$

where v is the rate of reaction, and a and b the concentrations of A and B. The rate is therefore proportional to the product of a and b

$$v \propto ab \tag{26}$$

(you may ask why the product of concentrations and not the sum: think of it as a matter of chance encounters between A and B. The probability of a collision between an A and a B depends on how much A *and* B there is, not on how much A irrespective of how much B, or how much B irrespective of how much A. When probabilities depend on each other like this, the combined probability is always the product of the individual probabilities).

In general, **reaction rate** is defined as minus the rate of change of reactant concentration, or plus the rate of change of product concentration. Translating this to the example above, the reaction rate tells us how fast the concentration of reactant A or the concentration of reactant B decreases with time, or how fast the concentration of the product A · B increases with time. The two rates will of course be the same, because for every molecule A and molecule B that disappear, one molecule of A · B appears. Mathematically the rate equals

$$v = -\frac{da}{dt} = -\frac{db}{dt} = \frac{d(a \cdot b)}{dt} \tag{27}$$

The reaction rate v thus has units of concentration·time^{-1}.

Remember that since we are considering an elementary reaction, the concentration of product A·B does not affect the rate (obviously, if we are considering the reverse reaction, the dissociation of A·B into A and B, its rate will be proportional to the concentration of A·B, and will be unaffected by A or B).

The above relationship between rate v and concentrations a and b can be transformed into a **rate equation** by inserting a proportionality constant, called the **rate constant**:

$$v = kab \tag{28}$$

The value of the rate constant is unique for every reaction. It is not, however, a fundamental constant, because it varies with environmental conditions such as temperature.

Imagine that in the above example we measure the reaction rate at different concentrations of a while keeping b constant. If we plot v against a then, because of the proportionality, we obtain a straight line through the origin. The slope of this line (kb, where b is fixed) is of fundamental importance as it tells us something about the reactivity of A and B. The steeper the slope at some fixed b, the more vigorous the reaction between A and B. The same argument holds when b is varied at fixed a.

The linear relationship between v and either a or b is called a **first-order** mathematical relationship (the order of any polynomial function is the highest exponent to which the variable is raised). This term has been adopted in the language of reaction kinetics: the above reaction is said to be first-order in a and first-order in b. In general, the **order of a reaction** with respect to some chemical species is the exponent to which the concentration of that species is raised to in the rate equation. The **overall order** of a reaction is the sum of all the exponents of the concentration terms in its rate equation (in our example the overall order is therefore two, and the reaction is called a second-order reaction: if both a and b are varied in the same proportion the rate curve would be parabolic). It should be clear that for elementary reactions the overall order can only be one (for dissociation and isomerisation reactions) or two (for association reactions).

5.2.1 Reaction order and sensitivity

In the light of the above, one may expect reaction order to be an integer, but in general, for ordinary chemical reactions, there is no such requirement, the reaction orders being complex functions of elementary orders. Reaction order is therefore an experimentally determined entity. Consider a reaction between two species A and B where the rate equation is of the form

$$v = ka^p b^q \tag{29}$$

and where p and q are the unknown orders. Taking logarithms on both sides we obtain

$$\ln v = \ln k + p \ln a + q \ln b \tag{30}$$

We have used natural logarithms (base $e = 2.718\ldots$), but we could also have used common logarithms with base 10. The logarithmic form of the equation immediately suggests a way of determining p and q experimentally. To obtain p we measure the rate v at fixed b for different concentrations of a. When $\ln v$ is plotted against $\ln a$ a straight line is obtained with slope p and an intercept on the $\ln v$-axis of $(\ln k + q \ln b)$. The inverse experiment where b is varied at fixed a yields q.

In this example we obtained straight lines when plotting $\ln v$ against $\ln(\text{concentration})$. This does not have to be so. In fact, for us as biologists, who virtually always study enzyme-catalysed reactions, the plot of $\ln v$ against $\ln s$, where s is substrate concentration, always yields a curve. This means that there is no fixed reaction order (no constant slope), but that the order (slope) varies with substrate concentration. Nevertheless, it is still possible to calculate the order at any given concentration as the value of the slope at that concentration. Now, if you recall your first-year calculus, you will remember that the slope of a curve at a given point is the first derivative of the function that describes the slope evaluated at that point. This leads to the most general definition of reaction order with respect to species a as

$$\frac{d \ln v}{d \ln a} \tag{31}$$

evaluated at a given a. (Because v is a function of both a and b, the reaction order should, strictly speaking, be a partial derivative $\left(\frac{\partial \ln v}{\partial \ln a}\right)_b$. Remember that the subscript outside the brackets lists the variables that are kept constant—here b—while a is varied). Don't let the symbolism put you off. It is just a short-hand notation for the slope of the curve obtained when plotting $\ln v$ against $\ln a$ at constant b.

The concept of reaction order is extremely important and we will return to it later in the course, when discussing metabolic control in a system of coupled reactions.

You may feel uncomfortable with this definition of reaction order as the slope of the curve of v versus a in log-log space; in fact, at a deeper level, you may feel uncomfortable with the introduction of the concept of logarithms into the discussion (in the days before calculators logarithms were introduced as a mathematical trick for calculation; you know, adding the logs of two quantities is the same as multiplying those quantities; this easily leads to the mistaken view that as we now use calculators we can safely ignore logs). The one answer often heard as a counter to this discomfort is: the definition of reaction order in terms of logarithms falls out of the mathematical derivation; just accept it and quit worrying. We distrust this type of reasoning. There must be a deeper and clearer explanation for why logarithms appear at all. We shall cover this topic extensively when we examine rate characteristics of enzyme-catalysed reactions in a following section. You will see that we shall also draw those rate characteristics as logarithmic graphs.

To conclude this section, let us focus more closely on the distinction between the terms "molecularity" of a reaction and "reaction order". The **molecularity** of a reaction has to with the number of molecules that react, which is given by the so-called stoichiometric coefficients of the reactants in the balanced reaction. In terms of the three elementary reactions, the isomerisation and dissociation reactions are unimolecular, while the association reaction is bimolecular. For these elementary reactions we have seen that the molecularity is equal to the overall reaction order. For any real chemical reaction this does not have to be so: reaction order is an experimentally determined entity and can in general not be obtained from the stoichiometric coefficients.

We have seen that the answer to the question "How fast does a reaction go?" is embodied in an experimentally determined rate equation. The main aim of reaction kinetics is to obtain such a rate equation for any reaction. Further on in this course we shall specifically discuss the special rate equations that describe enzyme-catalysed reactions.

5.3 The equilibrium constant—how far?

The second fundamental question to be asked about any reaction is "How far does a reaction go?". This question forms the starting point for what is known as *chemical thermodynamics* or, better, *chemical energetics*. We have already stated that all reactions are in principle reversible. How do we reconcile this statement with what we discussed above? The statement implies that every reaction can be thought of as a combination of a forward reaction and a reverse reaction, each described by an irreversible rate equation. For example, using so-called *mass-action kinetics*, the reversible reaction $\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{C}$ can be seen to be a combination of the forward reaction

$$\mathrm{A} + \mathrm{B} \rightarrow \mathrm{C} \qquad \text{with rate equation} \qquad v_f = k_f ab \tag{32}$$

and the reverse reaction

$$\mathrm{C} \rightarrow \mathrm{A} + \mathrm{B} \qquad \text{with rate equation} \qquad v_r = k_r c \tag{33}$$

The *net* rate of reaction is the *difference* between the forward and reverse rates

$$v = v_f - v_r = k_f ab - k_r c \tag{34}$$

If one starts off with only A and B and no C, then the reaction initially can only go forward. With only C and no A or B the reaction can initially only go backward. It follows that there *must always* be some point in between these two extremes where the concentrations of reactants and products are such that the forward and reverse reaction rates are equal so that the net rate is zero. This point is called the **dynamic equilibrium point** ("dynamic" because the forward and reverse reactions are still going on even though the concentrations of reactants and products are constant). This also implies that, when a reaction proceeds in isolation, the net direction of reaction is always towards the equilibrium point. No reaction can spontaneously move away from equilibrium

At equilibrium, therefore:

$$k_f (a)_{eq} (b)_{eq} - k_r (c)_{eq} = 0 \tag{35}$$

so that

$$\frac{k_f}{k_r} = \frac{(c)_{eq}}{(a)_{eq}(b)_{eq}} = K_{eq} \tag{36}$$

The subscripts *eq* refer to *equilibrium concentrations*. The concentrations of A, B and C in Eqs. 35 and 36 do not refer to any arbitrary condition, but *only to the case where the reaction is in equilibrium.*

The ratio of forward and reverse rate constants is called the **equilibrium constant**, K_{eq}, and its value is arguably the most important piece of information that one can have about a reaction. Although the ratio of forward and reverse rate constants is an important way of describing the equilibrium constant, it is the function given above in terms of equilibrium reactant and product concentrations which is the most useful in practice.

The first thing to notice is that, as defined above, the equilibrium constant for the reaction $A + B \rightleftharpoons C$ has units, namely that of (concentration)$^{-1}$. The equilibrium constant for the same reaction written in the opposite direction $C \rightleftharpoons A + B$ is of course the inverse of that for the original direction, and has units of concentration. Here it is important to note that the so-called thermodynamic equilibrium constant (see Section 5.4) looks the same as the one above but is always dimensionless because each concentration term is divided by a standard concentration of 1 M.

To end this section we expand the expression for the equilibrium constant to that of the general reaction

$$mA + nB \rightleftharpoons pC + qD \tag{37}$$

where m, n, p, and q are the **stoichiometric coefficients**. From the mass-action rate equations for the forward and reverse reactions

$$v_f = k_f a^m b^n \quad \text{and} \quad v_r = k_r c^p d^q \tag{38}$$

and using the equilibrium condition $v = v_f - v_r = 0$ we obtain

$$K_{eq} = \frac{(c)_{eq}{}^p (d)_{eq}{}^q}{(a)_{eq}{}^m (b)_{eq}{}^n} \tag{39}$$

Note that the exponents in the equilibrium expression are stoichiometric coefficients that describe the molecularity of the reaction. The above derivation using forward and reverse rate equations may have given the impression that they should be reaction orders, but we have already seen that molecularity does not necessarily equal reaction order. The form of the equilibrium expression should actually not be derived from rate equations as above, but from basic thermodynamic considerations where kinetic parameters such as reaction orders have no role to play. We will focus in more detail on the relationship between thermodynamic quantities (Gibbs energy) and the equilibrium constant in Section 5.4.

5.3.1 The mass-action ratio and the distance from equilibrium

With this background we can now refine our original question of "How far?" to "For a given set of concentrations of reactants and products, how far is the reaction from equilibrium and on which side?" For anyone studying living systems this question is of great importance because the hallmark of living systems is that they are out of equilibrium—being in equilibrium means being dead. The classical view of metabolic regulation sets great store by the classification of reactions into near-equilibrium and far-from-equilibrium (also referred to as non-equilibrium) reactions. Where to draw the line has been the subject of much (and rather futile) discussion. Let us rather derive an unambiguous quantitative measure for distance from equilibrium which also gives the direction in which the reaction proceeds.

The relationship that we seek must have something to do with the relationship between the forward and reverse rates: the greater the difference between them, the further away from equilibrium. Although the algebraic difference between forward and reverse rate (i.e., the net rate), may seem to be a quantity that measures the distance from equilibrium, there is a much better measure, namely the ratio of forward and reverse rates (which is dimensionless):

$$\frac{v_r}{v_f} = \frac{k_r c}{k_f ab} = \left(\frac{c}{ab}\right) \Big/ K_{eq} \tag{40}$$

Note that the concentrations a, b, and c can have any value. In the special case where they are equilibrium concentrations then the fraction c/ab *is* the equilibrium constant and the ratio v_f/v_r is equal to one (which of course it must be as at equilibrium the two rates are equal).

A quantity such as c/ab in the above equation is so important that it has been given a special name, the **mass-action ratio**, usually symbolised by Γ (capital Greek gamma). **Note that Γ is always exactly the same algebraic function of concentrations that one finds in the expression for the equilibrium constant**. In fact, the equilibrium constant is just a special value of the mass-action ratio, namely that when the concentrations are equilibrium concentrations (Fig. 12 and the following section explains this in more detail).

We can therefore write this equation as

$$\frac{v_r}{v_f} = \frac{\Gamma}{K_{eq}} = \rho \tag{41}$$

where ρ is also called the **disequilibrium ratio**.

5.4 The relationship between Gibbs energy, the mass action ratio and the equilibrium constant

In the Biochemistry 244 course you were introduced to the concept of change in Gibbs energy (ΔG) as the driving force for any chemical reaction. Recall that for any process (and hence, also for chemical reactions) the change in Gibbs energy determines whether that process will occur spontaneously:

- $\Delta G < 0$ — the process proceeds from what we regard as the initial state to the final state (for a chemical reaction from reactants to products).
- $\Delta G = 0$ — the process is at equilibrium.
- $\Delta G > 0$ — the process cannot proceed from what we regard as the initial state to the final state, but it rather occurs in the reverse direction (for a chemical reaction from products to reactants).

The above can be summarised in the following important statement:

> *The change in Gibbs energy of a process at constant pressure and temperature is an indication of the tendency of that process to occur spontaneously in a particular direction.*

The quantity ΔG provides a new way for interpreting the thermodynamic driving force for a process—the further away ΔG is from zero, the greater is the driving force for that process to occur (forward if $\Delta G < 0$, backward if $\Delta G > 0$).

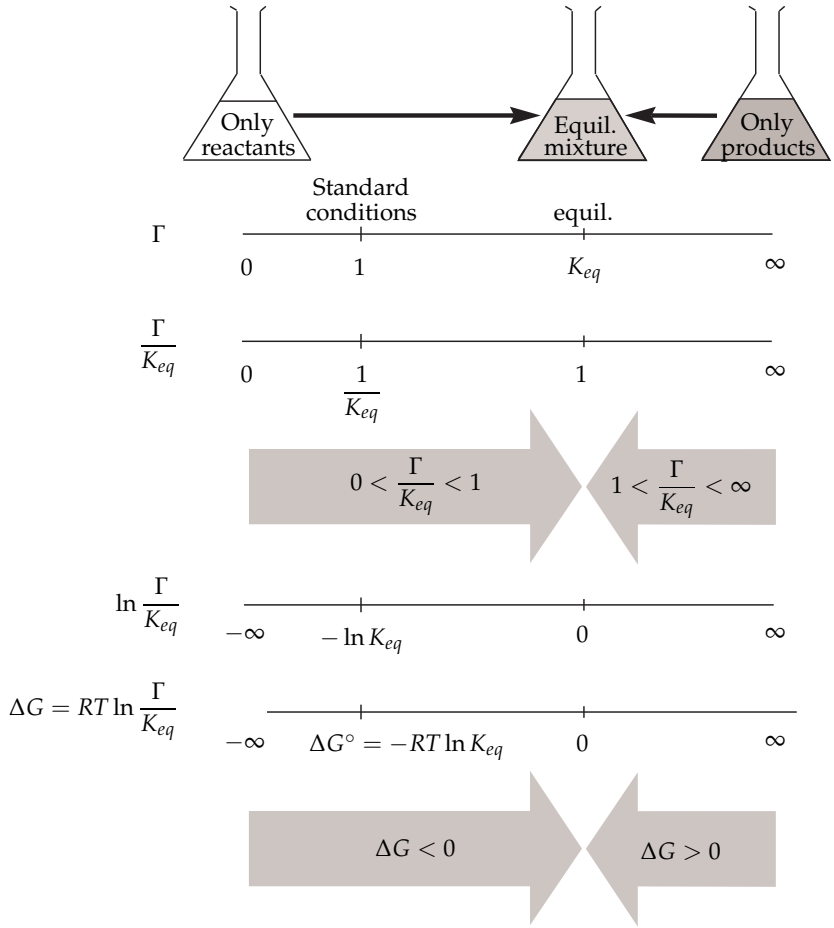

Figure 12: *How the disequilibrium ratio measures the "distance" from equilibrium and the direction in which a reaction proceeds from any specified value of the mass-action ratio.* The relationship between the disequilibrium ratio and the Gibbs energy of a reaction is also shown.

Clearly, the disequilibrium ratio Γ/K_{eq} and ΔG must be related in some way, since both give the same kind of information, namely the distance from equilibrium and the direction of reaction. This relationship is derived in an informal way in Fig. 12. Let us go through the figure:

From left to right the figure describes a continuous scale of reaction conditions, from a situation where there are only reactants (i.e., $\Gamma = 0$) to one where there are only products (i.e., $\Gamma = \infty$). Any other mixture of reactants and products will give a particular Γ which lies in between these two poles (note that any one Γ-value can be given by infinitely many combinations of reactant and product concentrations—work this out for yourself). Nevertheless, somewhere between the two extremes there must, for any reaction, be sets of reactant and product concentrations which, when inserted in the expression for Γ, yield the equilibrium constant (this is indicated on the Γ-scale; the position is arbitrary and will differ for different reactions). We can transform the Γ-scale into one for Γ/K_{eq} by dividing throughout with K_{eq}. On this scale the equilibrium point is at 1. The shaded arrows show the conditions that lead to the reaction going forward (left-to-right) or backward (right-to-left). The bottom part of the figure shows how Γ/K_{eq} can be transformed into ΔG. First, the Γ/K_{eq} scale is made logarithmic: now the equilibrium point is 0 (remember that $\ln 1 = 0$). The function $\ln(\Gamma/K_{eq})$ is still a dimensionless quantity, but if it is multiplied by the product RT then it acquires the units of energy

J.mol^{-1} (R is the universal gas constant with units J.mol^{-1}.K^{-1} and T is absolute temperature with units K, Kelvin). The shaded arrows show how Γ/K_{eq} and ΔG give the same information. In fact, the figure makes it quite clear that, for a chemical reaction, Γ/K_{eq} and ΔG are essentially the same thing:

$$\Delta G = RT \ln \frac{\Gamma}{K_{eq}} \tag{42}$$

This equation provides an excellent summary of ΔG as a measure for the driving force of a reaction towards equilibrium from a specified set of non-equilibrium conditions. The disequilibrium ratio Γ/K_{eq} indicates how far a reaction is away from equilibrium. ΔG is merely this term on a log-scale, multiplied by a constant to convert it to energy units.

So far we have considered only the equilibrium point on the scales in Fig. 12. There is, however, another very important point of reference, namely that where all the reactant and product concentrations are in their **standard state** (a concentration of 1 M for dissolved substances; a pressure of 1 atm for gases). The standard state value for Γ is therefore 1 (which could have units), and eq. 42 becomes:

$$\Delta G^\circ = RT \ln \frac{1}{K_{eq}} = -RT \ln K_{eq} \tag{43}$$

The term ΔG° therefore refers the Gibbs energy change measured under standard state conditions (the superscript $^{\circ}$ is the symbol used to denote this). It describes the driving force towards equilibrium when proceeding from standard conditions.

There is however, one important difference between the equilibrium constant as we have defined it before (eq. 39) and the one used in eq. 43, which is called the **thermodynamic equilibrium constant**. Depending on the number of concentration terms in the numerator and denominator, the earlier one may have units; the thermodynamic one is always dimensionless. This follows from the fact that the Γ-value of 1 in the standard state will have the same units as the equilibrium constant, so that the units in the expression $1/K_{eq}$ cancel. In effect what this means is that each concentration term in the equilibrium constant expression, say $(x)_{eq}{}^y$, is divided by $(1\text{M})^y$.

eq. 43 enables us to use thermodynamic data (in particular standard Gibbs energies) to calculate the equilibrium constant for any real or imaginary chemical reaction.

In summary, just as ΔG is just a reformulation of Γ/K_{eq} for a chemical reaction, ΔG° is just a reformulation of the equilibrium constant. Note, however, that although ΔG° is related to the equilibrium constant, it describes the Gibbs energy for *standard conditions, not for equilibrium conditions* (where the Gibbs energy is always 0).

Using eq. 43 we can now rewrite eq. 42 as follows:

$$\Delta G = RT \ln \Gamma - RT \ln K_{eq} = RT \ln \Gamma + \Delta G^\circ \tag{44}$$

This is the form of the equation that you have probably encountered in previous courses on energetics. Let us write it out for the case of the general balanced reaction given previously in eq. 37.

$$m\text{A} + n\text{B} \rightleftharpoons p\text{C} + q\text{D} \tag{45}$$

Using the full expression for Γ,

$$\Gamma = \frac{(c)^p (d)^q}{(a)^m (b)^n} \tag{46}$$

the change in Gibbs energy is given by:

$$\Delta G = \Delta G^\circ + RT \ln \frac{(c)^p (d)^q}{(a)^m (b)^n} \tag{47}$$

The Gibbs energy change for a reaction under any given conditions is thus given by a term for all reagents and products in their standard state (ΔG°), and a term that reflects the concentrations in the system under the specific conditions considered.

Remember that when the reaction is **in equilibrium**,

$$\Delta G = 0. \tag{48}$$

Hence, as before (compare eq. 43), it follows that

$$\Delta G^\circ = -RT \ln \frac{(c)_{eq}{}^p (d)_{eq}{}^q}{(a)_{eq}{}^m (b)_{eq}{}^n} \tag{49}$$

Remember that ΔG° indicates the direction of a reaction only in the special case where $\Gamma = 1$ (standard conditions). Under any other conditions, the corresponding value of ΔG has to be used. We emphasise this because ΔG° and ΔG are *often confused* in biochemistry; because ΔG° can be measured more easily, it is (wrongly!) used as the measure for the tendency of a reaction to occur. From the above it should be clear that, for conditions differing from the standard state (such as those present in living cells), the values of ΔG and ΔG° will differ; they will sometimes even differ in sign.

The relationship between ΔG, ΔG°, K_{eq} and the direction of the reaction is illustrated in Figs. 13 and 14. The curves for both figures were calculated for the simple reaction A $\rightleftharpoons$ B. The abscissa shows the mole fraction of B in the mixture, while the vertical scale indicates the Gibbs energy for a system containing both A and B, with a total amount A + B of 1 mol. The curve thus describes the relative Gibbs energy of such a system as a function of the changing mole fractions of its components. The slope of the curve at any point equals ΔG (in J mol^{-1}) for the reaction A $\rightleftharpoons$ B. One might imagine the reaction as a ball rolling inside the curve towards the direction of equilibrium (at the bottom where the slope equals zero); the tendency to roll becomes greater and greater the further the ball is away from equilibrium (higher up against the curve), because the slope of the curve is constantly increasing.

In Fig. 13 the equilibrium constant K_{eq} equals 1. ΔG°, the slope of the curve when $\Gamma = 1$ (and thus, when the mole fraction equals 0.5), equals zero here because at equilibrium the concentrations of reactant and product are equal so that $\Gamma = 1$ (if $K_{eq} = 1$, the mass action ratio of the reaction mixture at equilibrium is the same as that under standard conditions: $0.5/0.5 = 1/1 = 1$). The slope drawn at arbitrary mole fraction a illustrates the fact that the slope of the curve at any point equals the molar Gibbs energy change of the reaction for that composition of the reaction mixture.

Fig. 14 illustrates curves for two reactions with equilibrium constants different from 1. When $K_{eq} = 4$, the system is at equilibrium if the mole fraction of B equals 0.8. If the mole fraction is less than 0.8, the slope is negative and the reaction from A to B is thermodynamically possible. The standard Gibbs energy change ΔG° is, as before, the slope at a mole fraction of 0.5, i.e. when $\Gamma = 1$.

The other curve in Fig. 14 shows the reciprocal case where $K_{eq} = 0.25$ and ΔG° is positive.

Because the relationship between Γ and ΔG of a reaction only has one degree of freedom, the whole curve is specified by the value of the slope at any value of the mole fraction (or any value of Γ for the more general case of a reaction having more than one substrate and/or product). It is for

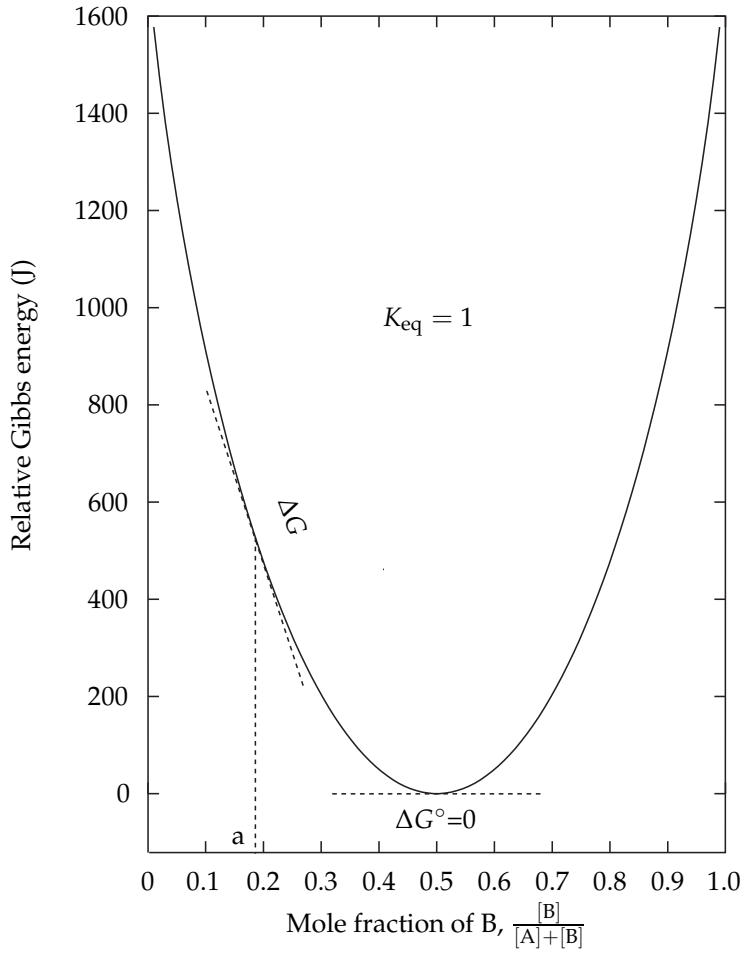

Figure 13: *Relative Gibbs energy of a system containing the compounds A and B in a total amount of 1 mol, plotted as a function of the mole fraction of B.* ΔG, the molar change in Gibbs energy for the reaction at an arbitrary composition of the A-B mixture, is given by the slope of the curve at the point referring to that reaction mixture composition (e.g., at point *a*).

this reason that $\Delta G°$ values are so convenient. The value of $\Delta G°$ by itself is not so significant, but it can be used to calculate the equilibrium point (the minimum of the curve), as well as the value of ΔG (the slope of the curve) at any other value of Γ: the further removed the standard state is from equilibrium, the greater is the slope at that point (the value of $\Delta G°$).

Remember that G itself does not have an absolute value, so that the position of zero on the vertical axis is arbitrary. For convenience's sake, in order to compare the curves, we assigned a value of zero to the relative Gibbs energy of the equilibrium mixture (the minimum of the curve).

5.5 Coupled reactions

Two reactions are said to be coupled when the product of one reaction serves as a reactant for the next reaction; this substance is then called the **common intermediate**. Such a situation is common in metabolic pathways. An example is:

$$A \rightleftharpoons B \rightleftharpoons C$$

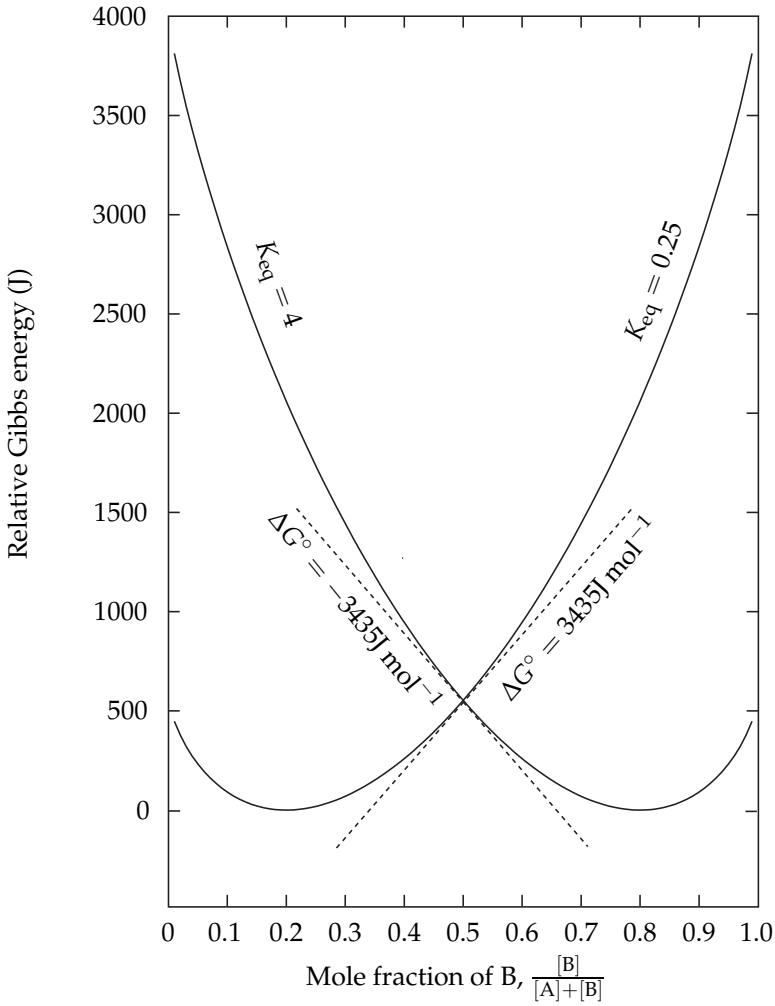

Figure 14: *The same as Fig. 13, except that the relative Gibbs energy changes are shown for the cases where* $K_{eq} = 0.25$ *and* $K_{eq} = 4$. The standard change in Gibbs energy, ΔG°, is the slope of the curve at a reaction mixture composition that gives a value of 1 for the mass-action ratio Γ.

where B is the common intermediate. For such a system of coupled reactions to be in equilibrium, each individual reaction has to be in equilibrium.

Therefore, if K_{eq1} and K_{eq2} are the two equilibrium constants,

$$K_{eq1} = \frac{(b)_{eq}}{(a)_{eq}} \quad \text{and} \quad K_{eq2} = \frac{(c)_{eq}}{(b)_{eq}} \tag{50}$$

where a, b, and c are equilibrium concentrations.

For the sequence as a whole one can also write an equilibrium constant K_{eq12} as

$$K_{eq12} = \frac{(c)_{eq}}{(a)_{eq}} \tag{51}$$

It follows that the overall equilibrium constant must be the *product* of the individual equilibrium constants:

$$K_{eq1}K_{eq2} = \frac{(b)_{eq}}{(a)_{eq}}\frac{(c)_{eq}}{(b)_{eq}} = \frac{(c)_{eq}}{(a)_{eq}} = K_{eq12} \tag{52}$$

We know, however, that the equilibrium constant is related to the standard Gibbs energy change for a reaction (ΔG°). For the coupled system, we have from the definition of ΔG° (eq. 43):

$$\Delta G_1^\circ = -RT \ln K_{eq1} \tag{53}$$
$$\Delta G_2^\circ = -RT \ln K_{eq2} \tag{54}$$
$$\Delta G_{12}^\circ = -RT \ln K_{eq12} \tag{55}$$

Since $K_{eq12} = K_{eq1}K_{eq2}$, we can derive the following by taking logarithms on both sides and multiplying by $-RT$ throughout:

$$-RT \ln K_{eq12} = -RT \ln K_{eq1} - RT \ln K_{eq2} \tag{56}$$

Hence,

$$\Delta G_{12}^\circ = \Delta G_1^\circ + \Delta G_2^\circ \tag{57}$$

This relationship emphasises an important fact: ΔG°-values can be added, so that if ΔG° is known for two reactions, we can calculate, by addition, the ΔG° of the net reaction when the two reactions are coupled.

This additivity is of course not only a property of ΔG°-values, but of any ΔG-values in general.

The following example illustrates an important point which can easily be overlooked, i.e., two reaction series with the same global overall K_{eq} (and thus the same overall ΔG°) *do not necessarily lead to the same equilibrium concentration of the final product.* Consider the following:

If the initial concentration of reactant A is 1 M (with that of B and C both zero), which of the following two coupled reaction series will lead two the greatest equilibrium concentration of the product C? (a) series 1; (b) series 2; or (c) both series lead to the same equilibrium concentration of C.

- Series 1

$$\begin{array}{ccccccccc} \mathrm{A} & \rightleftharpoons & \mathrm{B} & \Delta G^\circ & = & +18,85\,\mathrm{kJ\ mol^{-1}}; & K_{eq} & = & 5\times10^{-4} \\ \mathrm{B} & \rightleftharpoons & \mathrm{C} & \Delta G^\circ & = & -18,85\,\mathrm{kJ\ mol^{-1}}; & K_{eq} & = & 2\times10^{3} \end{array}$$

- Series 2

$$\begin{array}{ccccccccc} \mathrm{A} & \rightleftharpoons & \mathrm{B} & \Delta G^\circ & = & -18,85\,\mathrm{kJ\ mol^{-1}}; & K_{eq} & = & 2\times10^{3} \\ \mathrm{B} & \rightleftharpoons & \mathrm{C} & \Delta G^\circ & = & +18,85\,\mathrm{kJ\ mol^{-1}}; & K_{eq} & = & 5\times10^{-4} \end{array}$$

The sum of the ΔG° values (or the product of the K_{eq} values) is the same for both reactions ($\Delta G^\circ = 0$ and $K_{eq} = 1$). Perhaps your immediate response is that as a result of this the equilibrium concentration of C should be the same in both cases. However, you have to distinguish carefully between an equilibrium *concentration* and an equilibrium *concentration ratio*. Although the final *ratio* $(c)_{eq}/(a)_{eq}$ needs to be the same for both series, the equilibrium concentration $(c)_{eq}$ differs between the two cases. In series 1, approximately 49.99 % of the A that was originally present is converted to C. In series 2, however, the equilibrium levels of A and C are each only approximately 0.05 % of the original a, while 99.9 % accumulates as B. Evidently, series 1 converts A to C more efficiently than series 2.

To generalise: a reaction with a low K_{eq} for A $\rightleftharpoons$ B can thus be effectively "pulled over" to C by a following reaction B $\rightleftharpoons$ C with a high K_{eq}; however, a high K_{eq} reaction A $\rightleftharpoons$ B cannot "push over" a low K_{eq} reaction B $\rightleftharpoons$ C to C.

5.6 Kinetic and energetic aspects of reaction rate

We have seen how the equilibrium constant for a chemical reaction is related to ΔG° and how the driving force ΔG is related to the disequilibrium ratio Γ/K_{eq}. We have also seen that the rate of a reaction depends on the concentrations of its reactants and products, as well as the forward and reverse rate constants k_f and k_r (see eq. 34). How can we unite these thermodynamic and kinetic aspects of reaction rate?

In principle, the rate of *any* reversible reaction can be written in a form where the *kinetic aspects are clearly separated from the thermodynamic aspects.* Consider a possible rate equation for the reaction $\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{C} + \mathrm{D}$:

$$v = k_f ab - k_r cd \tag{58}$$

When we factor out the forward rate term we obtain

$$\begin{aligned} v &= k_f ab \left(1 - \frac{k_r cd}{k_f ab}\right) && (59)\\ &= k_f ab \left(1 - \frac{1}{K_{eq}}\frac{cd}{ab}\right) \qquad \text{because} \quad \frac{k_r}{k_f} = \frac{1}{K_{eq}} && (60)\\ &= k_f ab \left(1 - \frac{\Gamma}{K_{eq}}\right) \qquad \text{because} \quad \frac{cd}{ab} = \Gamma && (61) \end{aligned}$$

A reversible rate equation is therefore always the product of a kinetic term, here $k_f ab$, and an energetic term $(1 - \Gamma/K_{eq})$. The kinetic term always has rate units of concentration.time^{-1}, and its functional form depends on the mechanism of the reaction: here it is very simple, but it can become quite complicated, e.g., for an enzyme catalysed reaction.

The energetic term $(1 - \Gamma/K_{eq})$, on the other hand, consists only of concentrations and the equilibrium constant; its *form is always the same* for any reaction. Clearly, when the reaction is in equilibrium ($\Gamma/K_{eq} = 1$) the energetic term is zero; otherwise, when $\Gamma/K_{eq} < 1$ the energetic term is positive, indicating a net reaction in the forward direction, or when $\Gamma/K_{eq} > 1$ it is negative, indicating a net reaction in the reverse direction. The greater the distance from equilibrium, the faster the rate of reaction.

5.7 The biochemical standard state

To end this section on energetics, a few words on the *biochemical* standard state. In biochemical text books you will often find the symbols $\Delta G^{\circ\prime}$ and K'_{eq}. The prime ($'$) behind the symbol refers to the *biochemical standard state,* which is defined as the normal chemical standard state, except that the pH = 7 ($[\mathrm{H}^+] = 10^{-7}$ M), with all other dissolved substances present at 1 M.

In the normal chemical standard state, all substances *including* $\mathrm{H}^+$are present at 1 M. Because pH 7 is more relevant to living systems than pH 0 ($-\log 1\mathrm{M}\ \mathrm{H}^+$), biochemists have defined the biochemical standard state.

The relationships between ΔG and Γ/K_{eq} hold regardless of whether the normal chemical standard state or the biochemical standard state is used. However, it is *important* which one is used: for ΔG° and K_{eq}, the reference H^+ concentration is taken as 1 M, whereas for $\Delta G^{\circ\prime}$ and K'_{eq} the reference H^+ concentration is 10^{-7} M.

6 Membrane transport

Every biological cell is enclosed by a membrane. The membrane is often used to define the borders of the system and all interactions with the extracellular environment proceed via the membrane. To fulfil its role as a functional separation between the cytoplasm and the environment, the membrane must act as a differential diffusion barrier to hold cellular components inside and keep unwanted molecules out. However, for a system to remain away from equilibrium (and alive), it must be at least partly open (i.e. allow for transport over the membrane) to the environment. In one of the following sections we shall see that all closed systems will eventually end up in equilibrium.

In this section we focus on transport of small molecules across a biological membrane. We will not study membrane structures in great detail, for this we refer to any biochemical text book on the subject. For this part of the module it is sufficient to know that a biological membrane is an asymmetric lipid bilayer, that is highly impermeable for ions. In addition to lipids, most membranes also contain proteins, either integral or peripheral, and these proteins are mobile due to fluidity of the membrane. If any of these terms are unclear, please refer to your biochemistry textbook. Here we will focus on the thermodynamics and kinetics of transport across membranes. We will distinguish between three different types of transport: passive and facilitated diffusion and active transport. In addition we will consider two types of substrates: uncharged molecules and ions.

6.1 Driving force for diffusion reactions

It might appear strange to consider diffusion as a reaction; since it is nothing more than random molecular motion there is no conversion of substrate to product, but merely a net transport of molecules. However, if we consider diffusion across a membrane we can distinguish between molecules at both sides of the membrane and could call them substrate (X_{out}) and product (X_{in}), even if they are the same molecular species. Such a diffusion reaction would be at equilibrium when the concentrations of X_{out} and X_{in} are equal, i.e. the equilibrium constant is 1 and $\Delta G^0 = 0$.

In the previous section we defined Gibbs free energy change (ΔG) of a reaction as the driving force: a reaction will only proceed if $\Delta G < 0$. For any given reaction: $X_{out} \rightarrow X_{in}$, the ΔG can be expressed as (refer to eq. 44, setting $\Delta G^0 = 0$):

$$\Delta G = RT \ln \frac{x_{in}}{x_{out}} \tag{62}$$

Thus, as long as the internal concentration of X (x_{in}) is lower than the external concentration, the Gibbs free energy change for the uptake of X is negative and if there is a mechanism the reaction will proceed spontaneously. For diffusion reactions we can distinguish two types of mechanism: passive diffusion and facilitated diffusion. The driving force for both types of mechanisms is the same; the Gibbs free energy difference for a reaction is independent of the mechanism via which the reaction proceeds.

6.2 Passive diffusion

Passive diffusion is the most basic form of transport; it is random diffusion of molecules down a concentration gradient without any protein involved as a catalyst for the reaction. One can distinguish a number of steps in such a transport reaction ($X_{out} \rightarrow X_{in}$):

1. dissolving of the molecule from the extracellular aqueous phase into the membrane phase,

2. diffusion across the membrane, and
3. dissolving from the membrane phase into the intracellular aqueous phase.

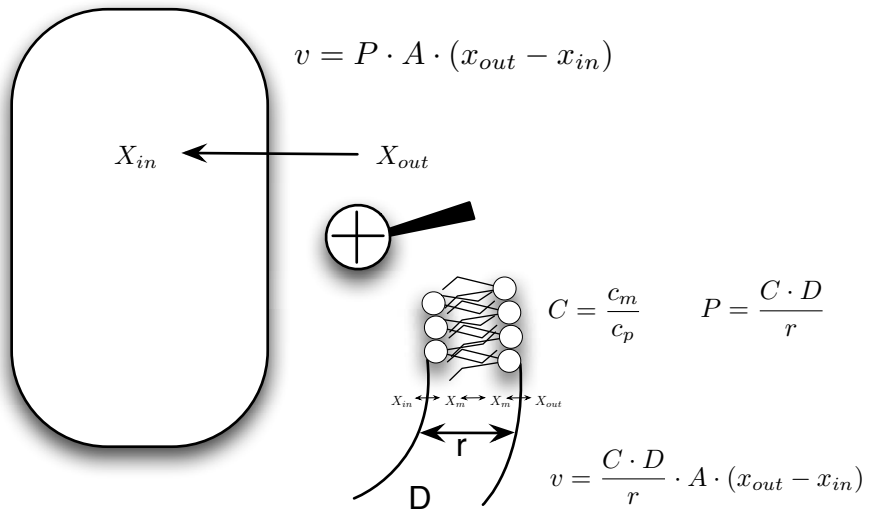

Figure 15: *Passive diffusion.* The passive diffusion process is illustrated for the uptake of molecule X. The uptake rate depends on the permeability coefficient P and the total membrane surface area A and is driven by the concentration gradient over the membrane. The detailed picture shows the dissolving and diffusion of X in the membrane. The permeability coefficient can be expressed as a function of the partition coefficient C, the diffusion coefficient D and the thickness of the membrane r.

The rate at which such a transport reaction proceeds is directly proportional to the concentration gradient across the membrane, and is described by Fick's law as follows:

$$v = P \cdot A \cdot (x_{out} - x_{in}) \tag{63}$$

with P a permeability coefficient (cm/s), A the area over which the transport takes place, i.e. the membrane surface area (cm^2), and v the transport rate (mol/s). Note that the units of the constants in the equation should be consistent. Since concentrations are usually expressed in molar units (i.e. mol/dm^3 or M), these need to be converted first to mol/cm^3, before they can be used in eq. 63.

The permeability coefficient is a phenomenological constant, describing the conductivity of a membrane for a certain molecule, and a more detailed description of the passive diffusion rate can be given with P as a function of a diffusion coefficient (D in cm^2/s), membrane thickness (r in cm) and partition coefficient (C, c_m/c_{aq}):

$$v = \frac{C \cdot D}{r} \cdot A \cdot (x_{out} - x_{in}) \tag{64}$$

Passive diffusion is often very slow compared to transport reactions that are catalysed. One of the reasons for this is that not many molecules readily dissolve in both aqueous solutions and in the hydrophobic membrane.

6.3 Facilitated diffusion

In the second type of passive transport reactions, facilitated diffusion, the reaction is catalysed by a transport protein. This is often a protein with a hydrophobic outer surface, such that it dissolves in the membrane, and a hydrophilic inner core that forms a channel through which it facilitates the diffusion of specific molecules. These transport proteins are often highly specific and involve binding of their substrates, they have a maximal rate (V_{max}) at which they can catalyse the transport reaction. The following rate equation can be used for facilitated diffusion, assuming that the binding constants for the substrate on both sides of the membrane are equal:

$$v = \frac{V_{max} \cdot (x_{out} - x_{in})}{K + x_{in} + x_{out}} \tag{65}$$

with K the dissociation constant (the lower its value the higher the affinity of the transporter for its substrate). Note that the reaction rate is zero when $x_{in} = x_{out}$, but that the rate is not proportional to the concentration gradient. The above equation reduces to the well known Michaelis-Menten equation when $x_{in} = 0$.

Thus, although the driving force for passive and facilitated diffusion is the same, the transport kinetics are very different. A comparison of uptake kinetics at a fixed internal concentration and varying external concentrations of the substrate is given in Fig. 16. Note the difference between the two plots: a linear relation for the passive diffusion reaction and a hyperbolic relation for the facilitated diffusion reaction. Facilitated diffusion reactions usually have much higher rates than the non-catalyzed reactions (this effect is even stronger in reality than indicated in the plot). Do you understand why the uptake rate becomes negative at low external substrate concentrations?

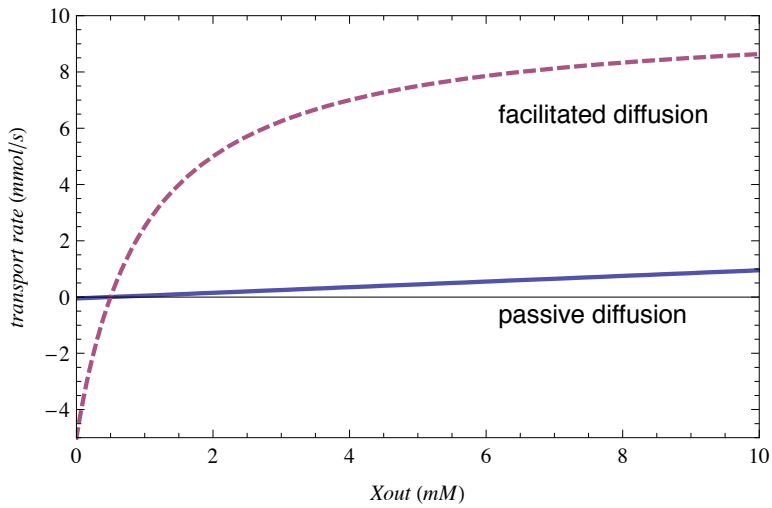

Figure 16: *Passive diffusion rate versus facilitated diffusion rate.* Uptake rates of x as a function of its extracellular concentration x_{out} are shown for a passive diffusion system and a facilitated diffusion system. The intracellular concentration x_{in} was constant at 0.5 mM. V_{max} and K for the facilitated diffusion system were 10 mmol/s and 0.5 mM respectively. Membrane area A and permeability coefficient P for the passive diffusion system were 1 cm^2 and 100 cm/s respectively (note that in reality these values are usually much smaller but were exaggerated here for demonstration).

6.4 Transport of ions and membrane potential

Thus far we have only considered transport of non-charged molecules for which the driving force can be directly related to the concentration gradient over the membrane. For ionic species an additional factor plays a role; the membrane potential due to the charge difference across the membrane. The electrostatic force due to charge separation contributes to the Gibbs free energy change of ion transport (in addition to the normal osmotic force i.e. chemical gradient). This is reflected in the so-called Nernst equation:

$$\Delta G = RT \ln \frac{x_{in}}{x_{out}} + ZF \cdot \Delta\psi \tag{66}$$

with Z the charge of the ion X, F the constant of Faraday (kJ/V/mol) and R the gas constant (J/K/mol).

If we assume that ions can move freely across a membrane, we can use this equation to calculate the membrane potential at which a system with a given ionic concentration gradient will be at equilibrium (i.e. will exhibit no nett flow of ions across a membrane due to the balancing of osmotic and electrical forces):

$$\Delta G = 0 \quad \Rightarrow \quad \Delta\psi = -\frac{RT}{ZF} \ln \frac{x_{in}}{x_{out}} \tag{67}$$

Example Assume a concentration ratio of 10 (in/out) of potassium ions ($Z = 1$). Calculate the membrane potential that would lead to equilibrium, using the Nernst equation (F = 96 485 J/V/mol, R = 8.31447 J/K/mol, T = 298.17 K).

$$\Delta\psi = -25.69 \ln 10 \tag{68}$$

$$= -59.16\,\text{mV} \tag{69}$$

Note that this potential is not the potential associated with the potassium ions themselves (more positive ions inside than outside) but with the membrane that prevents the flow of ions. We can therefore say the the inside of the membrane must be at a lower potential (more negative) than the outside to establish equilibrium in this specific scenario.

The Nernst equation is useful for systems where only one ionic species can move across the membrane, in which case the thermodynamic equilibrium state can be described by the equation.

In many biological systems several ionic species can move across the membrane with different permeabilities and then one needs to use the Goldman equation to calculate the membrane potential (note that this is different from the thermodynamic equilibrium for the individual ionic species as calculated using the Nernst equation). For C mono-valent positive ions and A mono-valent negative ions, the Goldman equation is given by:

$$\Delta\psi = -\frac{RT}{F} \ln \frac{\sum_i^C P_{x_i} x_{i_{in}}^+ + \sum_j^A P_{x_j} x_{j_{out}}^-}{\sum_i^C P_{x_i} x_{i_{out}}^+ + \sum_j^A P_{x_j} x_{j_{in}}^-} \tag{70}$$

For instance, in case we have a concentration gradient of Na^+, K^+, and Cl^- over a membrane, and we know the permeability for these ions, then we can calculate the resting potential with the following equation:

$$\Delta\psi = -\frac{RT}{F}\ln\frac{P_{Na^+}[\mathrm{Na}^+]_{in} + P_{K^+}[\mathrm{K}^+]_{in} + P_{Cl^-}[\mathrm{Cl}^-]_{out}}{P_{Na^+}[\mathrm{Na}^+]_{out} + P_{K^+}[\mathrm{K}^+]_{out} + P_{Cl^-}[\mathrm{Cl}^-]_{in}} \tag{71}$$

Verify yourself that with a permeability of zero for K^+ and Cl^-, the above equation reduces to the Nernst equation for Na^+. In the Goldman equation, relative permeabilities are often used for P (in contrast to the absolute values that are necessary when the P is used for calculations of transport rates as in eq. 63).

Example Typical concentration gradients observed over the axon membrane in neurons are: Na^+_{in} = 50 mM, Na^+_{out} = 400 mM, K^+_{in} = 400 mM, K^+_{out} = 20 mM, Cl^-_{in} = 60 mM, Cl^-_{out} = 560 mM, with relative permeabilities: $P_{K^+} = 1$, $P_{Na^+} = 0.04$ and $P_{Cl^-} = 0.45$. Calculate the rest potential where the concentration gradients are balanced, according to their permeabilities, by the membrane potential, using the Goldman equation (F = 96.485 kJ/V/mol, R = 8.31447 J/K/mol, T = 298.17 K).

$$\begin{aligned}
\Delta\psi &= -\frac{RT}{F}\ln\frac{P_{Na^+}[\mathrm{Na}^+]_{in} + P_{K^+}[\mathrm{K}^+]_{in} + P_{Cl^-}[\mathrm{Cl}^-]_{out}}{P_{Na^+}[\mathrm{Na}^+]_{out} + P_{K^+}[\mathrm{K}^+]_{out} + P_{Cl^-}[\mathrm{Cl}^-]_{in}} \\
&= -\frac{8.31447 \cdot 298.17}{96.485 \cdot 10^3}\ln\frac{0.04 \cdot 50 \cdot 10^{-3} + 1 \cdot 400 \cdot 10^{-3} + 0.45 \cdot 560 \cdot 10^{-3}}{0.04 \cdot 400 \cdot 10^{-3} + 1 \cdot 20 \cdot 10^{-3} + 0.45 \cdot 60 \cdot 10^{-3}} \\
&= -0.0601\,\mathrm{V}
\end{aligned}$$

Due to the impermeability of membranes for ions there is virtually no passive diffusion of ions and almost all ionic transport is facilitated, for instance via protein channels. We limit ourselves here to energetics of ion transport and will not go into any further details of the kinetics of ion transport.

6.5 Active transport and the coupling of processes

Diffusion transport processes of non-charged species run down a concentration gradient. By linking a transport process to another process with a large negative Gibbs free-energy change, it is possible to transport molecules against their concentration gradient. This process is referred to as active transport. Of course the overall Gibbs free-energy of the process must be negative, but by coupling reactions some of the partial reactions can have a positive Gibbs free-energy change. Important examples of such active transport processes are the ABC transporters, where the hydrolysis of ATP is the driving reaction, and proton symport, where the proton motive force is used to push reactions.

Active transport processes are always enzyme-catalysed and their kinetics can be quite complicated (for an introduction to multi-substrate kinetics see the next section). Here we will limit ourselves to the thermodynamics of the coupled reactions. As we did for the transport of ions, we need to add the Gibbs free-energy of the different processes together to calculate the ΔG for the overall process.

Example If we consider the ABC transporter as depicted in Fig. 17, and assume a ΔG_{ATP} for ATP hydrolysis of -57 kJ/mol, then we can calculate what the maximal glucose gradient would be at which the transporter could still import glucose, assuming 100 % efficiency of coupling between the

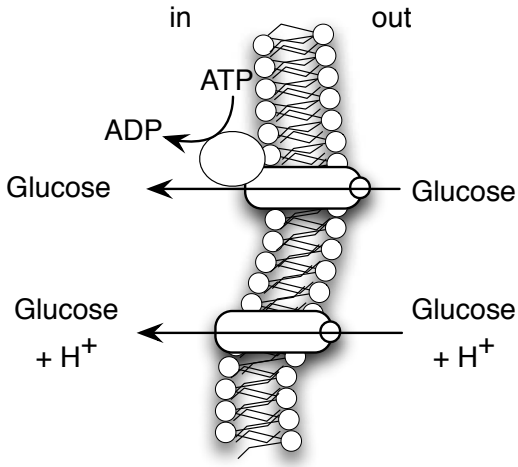

Figure 17: *Active transport.* Two examples of process coupling in active transport are shown: the ABC transporter and H^+-symport for glucose uptake. The ABC transporter couples the Gibbs free-energy change of ATP hydrolysis to the uptake of glucose, and the proton symport system links the electrochemical gradient of protons to the combined transport of a proton with a glucose molecule.

two processes and a stoichiometry of 1 mol of glucose transported per mol of ATP hydrolysed (i.e. 57 kJ/mol is available per mol of glucose transported):

$$\begin{aligned} \Delta G_{Glc_{up}} &= RT \ln \frac{x_{in}}{x_{out}} \\ 57 \cdot 10^3 &= 8.31447 \cdot 298.17 \cdot \ln \frac{x_{in}}{x_{out}} \\ \frac{x_{in}}{x_{out}} &= 1 \cdot 10^{10} \end{aligned}$$

Thus, linking ATP hydrolysis to glucose uptake makes it possible to transport glucose against an enormous concentration gradient, while the overall process would still have a $\Delta G < 0$. When making calculations such as the above it is important to be very careful with units; always ensure that the units are consistent (i.e. for the above calculation $\Delta G_{\text{ATP hydrolysis}}$ was given in kJ/mol, while R is defined as J/mol; check that the units in the above equation are used correctly), and also that you use the correct sign ($+$ or $-$) for the ΔG values (why is the value $+57 \cdot 10^3$ used?).

A similar calculation can be performed for the proton symport transporter. First calculate the ΔG_{H^+}; note that you need to take a concentration gradient (typically given as a pH difference) and a membrane potential into account. These two components for the calculation of the proton motive force (another name for ΔG_{H^+}) are not always working in the same direction, for instance if the internal pH is lower than the external pH, the concentration gradient for H^+ would work against a negative membrane potential. The calculated ΔG_{H^+} is the maximal Gibbs free-energy that is available for glucose transport per mol of H^+ taken up (note that the stoichiometry of glucose/H^+ must be taken into account).

6.6 Modelling the nervous system: the classic Hodgkin-Huxley equations

Higher organisms have a necessity for communication between the different cell types and organs. Whereas an important part of this communication is fulfilled by the hormonal system (dependent on

the blood-stream), an additional faster system (up to 100 m/s) is necessary to respond effectively to changes in the environment. The nervous system forms a very complicated communication system, linking the sensory inputs (e.g. sight, sound, smell) via the brain to various other cell types (e.g. muscle, heart).

Signals are carried across the nervous system via neurons. These are specialised cells, consisting of a cell body, an axon and dendrites. The signals are electrical in nature and occur as transient changes in electrical potential differences across the neuronal membrane.

6.6.1 Building the model

For a neuronal cell we need to take three ions into consideration each with their own permeability and concentration gradient. For a typical neuron at rest we find the following concentrations of ions and permeabilities:
Axon inside: 50 mM Na^+, 400 mM K^+, 60 mM Cl^-
Axon outside: 400 mM Na^+, 20 mM K^+, 560 mM Cl^-
Permeabilities: PK = 1; PNa = 0.04; PCl = 0.45.

Using the Goldman equation as shown above, one obtains the resting membrane potential of -60 mV which is in close agreement with the experimentally observed value. This implies that no ions move across the neuronal membrane if the inside of the membrane is at a potential of 60 mV lower than the outside.

Hodgkin and Huxley (J Physiology 1952) used the analogy to an electrical circuit when conceptualising their model for the ion currents over the neuronal membrane. Although originally constructed empirically without a molecular understanding of the mechanisms, the model has since been shown to reflect some of the mechanisms specifically regarding the behaviour of the ion channels.

In this model the phospholipid bi-layer is represented as a capacitor (since it accumulates charge as electrical potential changes across the membrane), the ionic permeabilities of the membrane as resistors (Na and K resistors are variable due to voltage-sensitivity of channels) and the electrochemical forces that drive the ionic currents as batteries (see Fig. 18).

Here the driving force that every ionic species feels (electromotive force) is due to the difference between the resting membrane potential (V_m) and the Nernst potential (also called reversal potential) of the specific species (E). The expression for an ionic current due to this force (with the outward direction defined as positive) is given by Ohm's law:

$$I = \frac{V}{R} = g(V_m - E) \tag{72}$$

With a resting membrane potential of -60 mV as calculated above, the K^+ ions, for example, feel very little force to move across the membrane since $E_K \approx -72\,\text{mV}$. However, if if ions are allowed to flow, K+ current will flow outwards (i.e. a positive current).

Expanding on this idea, if we apply an external stimulus current (I_{ext}) to the membrane, the total ionic current is as a result of the combined effect of all ionic species and can be written as

$$I_{ext}(t) = I_K(t) + I_{Na}(t) + I_L(t) + I_m(t) \tag{73}$$

For each component we can use the traditional interpretation of its circuit element to derive an equa-

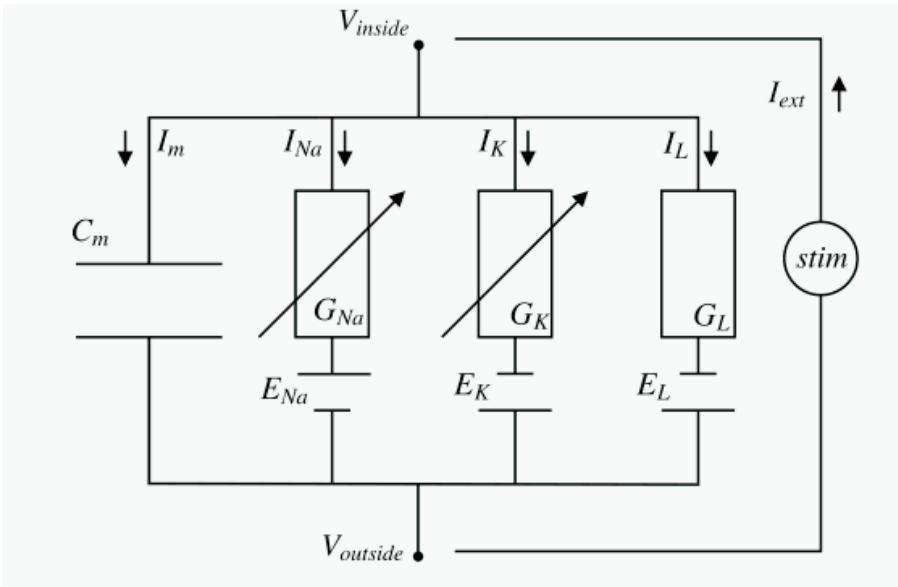

Figure 18: The circuit underlying the Hodgkin Huxley model. The branch featuring a capacitor represents die dielectric properties of the axonal membrane, the remaining branches its conductive properties. Resistors with an arrow represent voltage and time-dependent conductances that are related to the opening and closing of ion channels (see section 6.6.1 for details.)

tion for its associated current

$$\text{membrane capacitor} : I_m(t) = C_m \frac{dV_m}{dt} \tag{74}$$

$$\text{sodium channel} : I_{Na}(t) = \frac{V_{Na}}{R_{Na}} = g_{Na}\left(V_m(t) - E_{Na}\right) \tag{75}$$

$$\text{potassium channel} : I_K(t) = \frac{V_K}{R_K} = g_K\left(V_m(t) - E_K\right) \tag{76}$$

$$\text{leak channel} : I_L(t) = \frac{V_L}{R_L} = g_L\left(V_m(t) - E_L\right) \tag{77}$$

$$\tag{78}$$

and write the total ionic current as

$$I_{ext}(t) = C_m \frac{dV_m}{dt} + g_{Na}\left(V_m(t) - E_{Na}\right) + g_K\left(V_m(t) - E_K\right) + g_L\left(V_m(t) - E_L\right). \tag{79}$$

The voltage-dependent conductances of the ionic channels are written as

$$g_{Na} = \overline{G}_{Na} m(t)^3 h(t) \tag{80}$$

$$g_K = \overline{G}_K n(t)^4 \tag{81}$$

where $\overline{G}$ represents the maximal conductance and $m(t),\ h(t),\ n(t)$ are phenomenological functions that describe the degree of openness of a channel. These functions are fitted to experimental data and

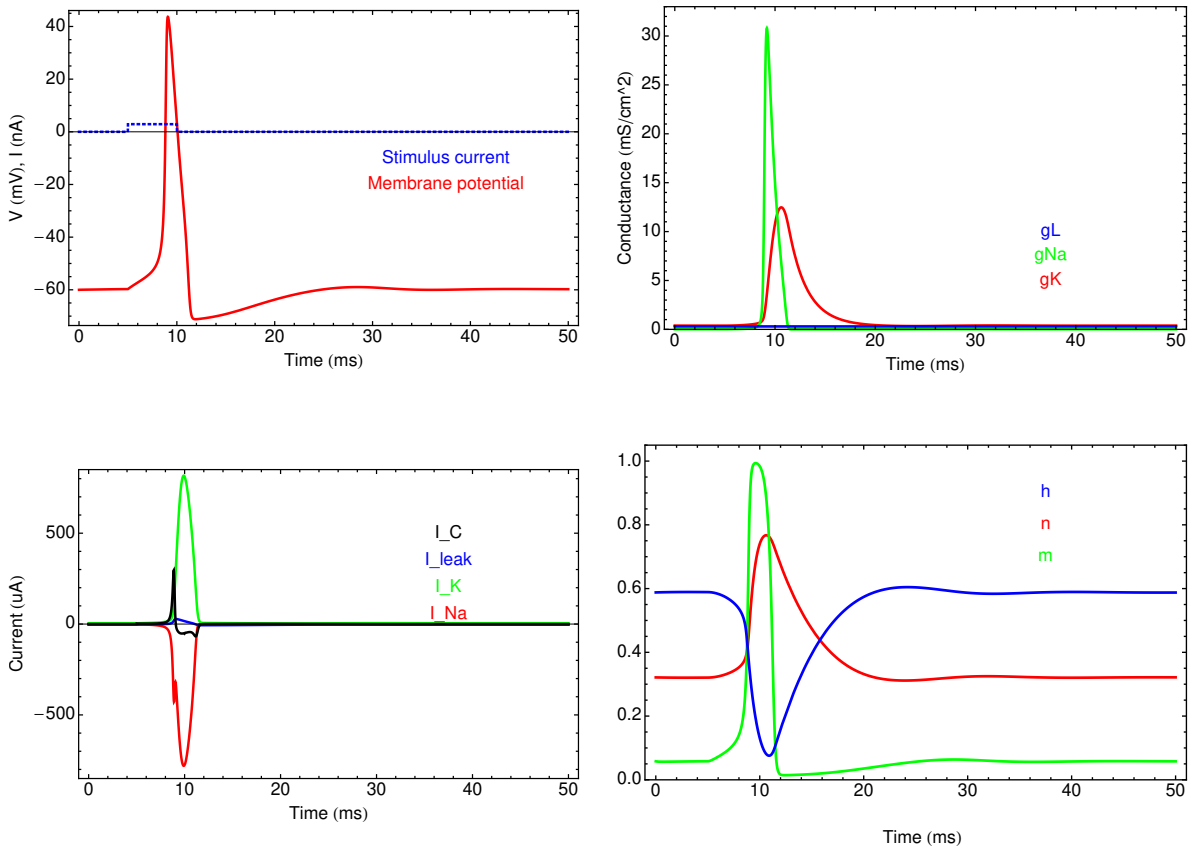

Figure 19: Generation of the action potential through the application of an external current stimulus.

are written in terms of voltage dependent rate constants (α and β) as

$$\frac{dn}{dt} = \alpha_n\left(V_m(t)\right)\left(1-n(t)\right) - \beta_n\left(V_m(t)\right)n(t) \tag{82}$$

$$\frac{dm}{dt} = \alpha_m\left(V_m(t)\right)\left(1-m(t)\right) - \beta_m\left(V_m(t)\right)m(t) \tag{83}$$

$$\frac{dh}{dt} = \alpha_h\left(V_m(t)\right)\left(1-h(t)\right) - \beta_h\left(V_m(t)\right)h(t). \tag{84}$$

Eqs 79 - 84 are the defining equations of the Hodgkin Huxley model and can be used to describe the generation and progression of an action potential along the neuronal membrane.

6.6.2 The action potential

The difference between the actual electrical potential and the equilibrium potential is not the same for Na^+, Cl^- and K^+ ions. Whereas for K^+ the actual potential is close to the equilibrium potential (see discussion above), for Na^+ there is a large difference. This makes the driving force for Na^+ ions to move into the neuronal cell much larger than the driving force for K^+ to move out. This difference in driving force is crucial in the depolarisation of the electric potential during the so-called action potential, the actual impulse that moves along the nerve cells.

A local depolarisation of about 20 mV (from the -60 mV resting potential to about -40 mV) is necessary for a dramatic effect on the conductivity of the voltage-gated ion channels (see Fig. 19). Upon

depolarisation these ion-channels (specific for K^+ and Na^+ ions) open up, leading to a rapid inflow of Na^+ ions, leading to a further depolarisation (and opening of ion-channels), until the electrical potential has reached values up to + 30 mV. At these high electrical potentials the Na^+ channels close and the continued efflux of K^+ leads to a restoration of the electrical potential. Na^+, K^+-ATPase and other channels restore the resting ion-gradients.

The action potential is rapidly passed along the axonal membrane. The Na^+ ions that are transported into the cell have to diffuse from somewhere else, leading to a depolarisation and a subsequent opening of the voltage-gated ion-channels in those adjacent regions, repeating the pattern described above.

Hodgkin and Huxley were the first to incorporate changes in permeability of the Na^+ and K^+ ions and put forward their model describing the action potential. They were rewarded a Nobel prize for their work in 1963.

7 Enzyme kinetics

One of the aims of this course is to arrive at a functional view of metabolic regulation. Metabolism occurs within living cells, which are surrounded by a plasma membrane. The previous section has dealt with the transport of small molecules and ions across this membrane.

We now return our attention to the reactions occurring *inside* the cell. In Section 5 we have looked at both thermodynamic (i.e. energetic) and kinetic driving forces for a chemical reaction. However, up to now we have only considered reactions that exhibit mass-action kinetics. Clearly, the situation in the living cell is more complex, as cellular reactions are catalysed by enzymes and exhibit more complex enzyme kinetics. In this section, we discuss how enzymatic catalysis affects the rate of chemical reactions; this is the field of *enzyme kinetics*.

7.1 How enzymes accelerate the rates of reactions

Enzymes have the power to accelerate the rates of reactions by orders of magnitude. For example, the hydrolysis of the metabolite fructose-1,6-bisphosphate occurs $10^{21}\times$ faster in the presence of the enzyme fructose-1,6-bisphosphatase than spontaneously in aqueous solution. How is this rate acceleration brought about?

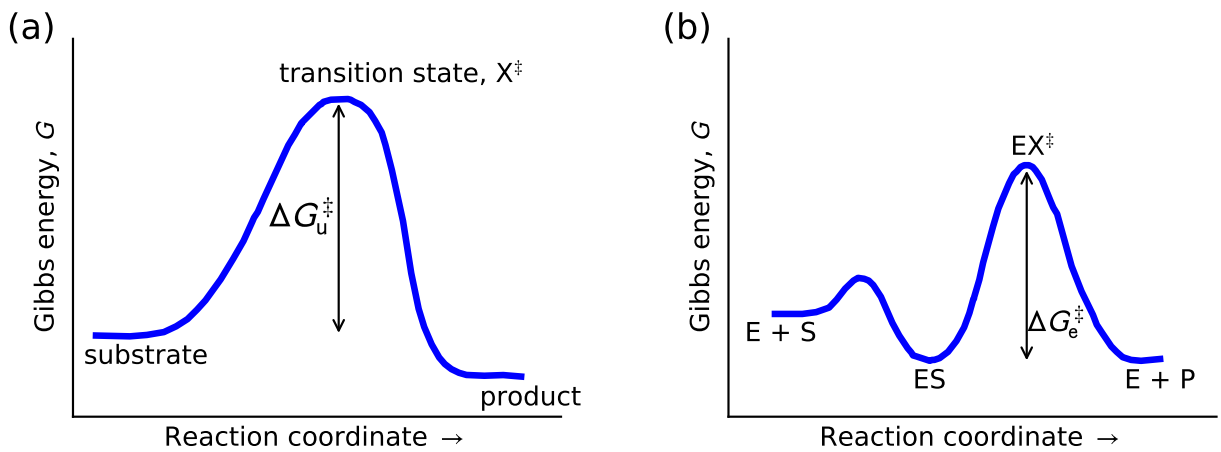

Figure 20: *Energy diagram for an uncatalysed (a) and an enzyme-catalysed (b) reaction.* The magnitude of the activation energy barrier is indicated by an arrow.

Fig. 20 shows the energy diagrams for an uncatalysed and an enzyme-catalysed reaction. This plots the Gibbs energy as a function of the reaction coordinate, i.e. how far the reaction has progressed on the reaction path. Every reaction proceeds via a so-called transition state (denoted by $X^{\ddagger}$), which can be viewed as a chemical structure in-between the substrate and the product. This transition state is energetically unfavourable, and the resulting energy barrier has to be crossed in order for the reaction to proceed. This energy barrier is called the *activation energy*, denoted by $\Delta G_u^{\ddagger}$ (u=uncatalysed).

An enzyme causes rate acceleration by *lowering the activation energy* (Fig. 20(b)): $\Delta G_e^{\ddagger} < \Delta G_u^{\ddagger}$ (e=enzyme-catalysed). The energy barrier that needs to be crossed is smaller, and hence the reaction proceeds more readily. Note that the Gibbs-energy difference between substrate and product is the

same as that between E+S and E+P. The enzyme *can therefore not change the equilibrium constant* of the reaction.

In order for the enzyme to catalyse a reaction, it needs to bind the substrate; hence, the Gibbs energy of the ES complex is lower than that of E+S. This energy difference is referred to as Gibbs energy of ES complex formation and is denoted by ΔG_{ES}. The enzyme also binds to the transition state ($\mathrm{EX}^{\ddagger}$), in fact, the lowering in activation energy is brought about by transition state stabilisation. The lowering in Gibbs energy due to transition state binding (difference between $\mathrm{X}^{\ddagger}$ and $\mathrm{EX}^{\ddagger}$) is denoted by ΔG_{b}.

7.1.1 Arrhenius theory, rate constants and binding constants

In 1889, the Swedish scientist Svante Arrhenius proposed the following formula to describe the temperature-dependence of a chemical reaction:

$$k = Ae^{-\Delta G^{\ddagger}/(RT)} \tag{85}$$

where k is the rate constant for the reaction, A is a proportionality constant also termed the pre-exponential factor, and $\Delta G^{\ddagger}$ is the activation energy. Apart from specifying the temperature-dependence (which we will not discuss further here), the equation relates the rate constant to the activation energy: the greater the activation energy (i.e. the taller the energy barrier), the smaller the rate constant and therefore the slower the reaction will proceed.

Returning to Fig. 20(a) and (b), the following relationship becomes clear:

$$\Delta G_{\mathrm{u}}^{\ddagger} + \Delta G_{\mathrm{ES}} = \Delta G_{\mathrm{e}}^{\ddagger} + \Delta G_{\mathrm{b}} \tag{86}$$

For catalysis to be efficient, the enzyme has to lower the activation energy, i.e. $\Delta G_{\mathrm{e}}^{\ddagger} < \Delta G_{\mathrm{u}}^{\ddagger}$. From Eq. 86,

$$\Delta G_{\mathrm{u}}^{\ddagger} - \Delta G_{\mathrm{e}}^{\ddagger} = \Delta G_{\mathrm{b}} - \Delta G_{\mathrm{ES}} \tag{87}$$

Therefore, $\Delta G_{\mathrm{u}}^{\ddagger} > \Delta G_{\mathrm{e}}^{\ddagger}$ implies that $\Delta G_{\mathrm{b}} > \Delta G_{\mathrm{ES}}$ (the transition state is stabilised more than the substrate). Using the Arrhenius equation (Eq. 85) it is possible to relate k_e (the rate constant for the enzyme-catalysed reaction) to $\Delta G_{\mathrm{e}}^{\ddagger}$ and k_u (the rate constant for the uncatalysed reaction) to $\Delta G_{\mathrm{u}}^{\ddagger}$.

In Section 5, we derived the relationship between the standard Gibbs energy difference for a reaction and the equilibrium constant (see Eq. 43). Turning to Fig. 20, we see that ΔG_{ES} can be related to K_s (the dissociation constant of substrate) and ΔG_{b} to K_T (the dissociation constant of the transition state from the enzyme):

$$\Delta G_{\mathrm{ES}} = -RT \ln K_s \quad \text{and} \quad \Delta G_{\mathrm{b}} = -RT \ln K_T \tag{88}$$

Exercise Derive the following relationship:

$$k_e/k_u = K_s/K_T \tag{89}$$

7.1.2 Destabilisation of the ES-complex

The fact that $\Delta G_{\mathrm{ES}} < \Delta G_{\mathrm{b}}$ implies that the ES-complex has to be destabilised. This is brought about by two competing effects (Fig. 21).

Binding of S to the enzyme has a stabilising effect that lowers the Gibbs energy by ΔG_{b}, much in the same way that the transition state is stabilised by binding. However, a destabilising effect counteracts this, which has two components:

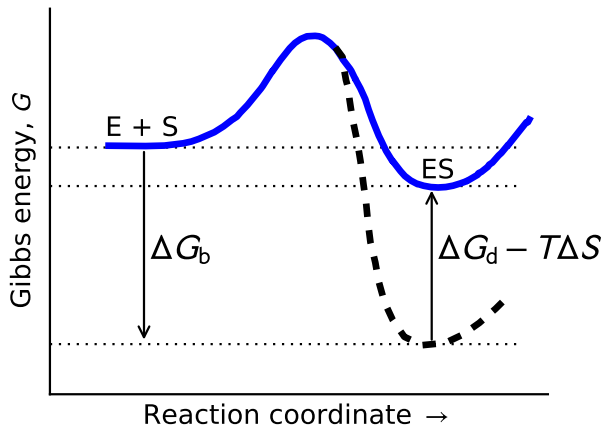

Figure 21: *Destabilisation of the ES-complex.* Binding of substrate to enzyme stabilises the complex (ΔG_b). This is counteracted by a destabilising effect which has a Gibbs energy component (ΔG_d) and an entropy component ($-T\Delta S$).

- *entropy loss* (quantified by $-T\Delta S$) due to binding of E to S. The ES complex is a highly ordered structure in constrast to S in free solution, where substrate and enzyme are free to undergo translational motion and have many more degrees of freedom of movement.
- *destabilisation* brought about by *interactions between the enzyme and the substrate* (quantified by ΔG_d). These include:

  strain and distortion Binding of substrate to enzyme might cause strain to be put on certain chemical bonds to distort them away from their original position. Enzymes are known to 'push' the chemical structure of the substrate so that it comes closer to the transition state, and in this way facilitate transition state formation.

  desolvation Substrate molecules in free solution are surrounded by a solvation shell of water molecules (refer to Biochemistry 214). When the substrate binds to the enzyme, the water molecules making up the solvation shell are removed in a process termed *desolvation*.

  electrostatic destabilisation When the substrate binds to the enzyme, negative (or positive) charges on the substrate may come into the proximity of charges of the same sign on the active site of the enzyme, leading to a repulsive interaction and destabilisation.

Destabilisation is necessary for catalysis (Fig. 22). If there were no destabilisation of ES (i.e. the enzyme would bind the substrate and transition state equally strongly), the Gibbs energy of ES would be so low that the activation energy would be the same, irrespective of whether S is enzyme-bound or not (Fig. 22(a)). Such a situation is referred to as a *thermodynamic pit*. In contrast, if ES is destabilised, the activation energy is lower for the enzyme-bound substrate than for the free substrate, so that catalysis is facilitated and rate acceleration is brought about (Fig. 22(b)).

Enzymes bind the transition state very strongly. To illustrate this, consider an enzyme with a typical K_s value of 10^{-4} M for its substrate. Assume now that the rate acceleration brought about by enzymatic catalysis ia 10^{16} (a typical value). From Eq. 89 we calculate that $K_T = 10^{-20}$ M. This is a tiny dissociation constant for the transition state, indicating extremely tight binding.

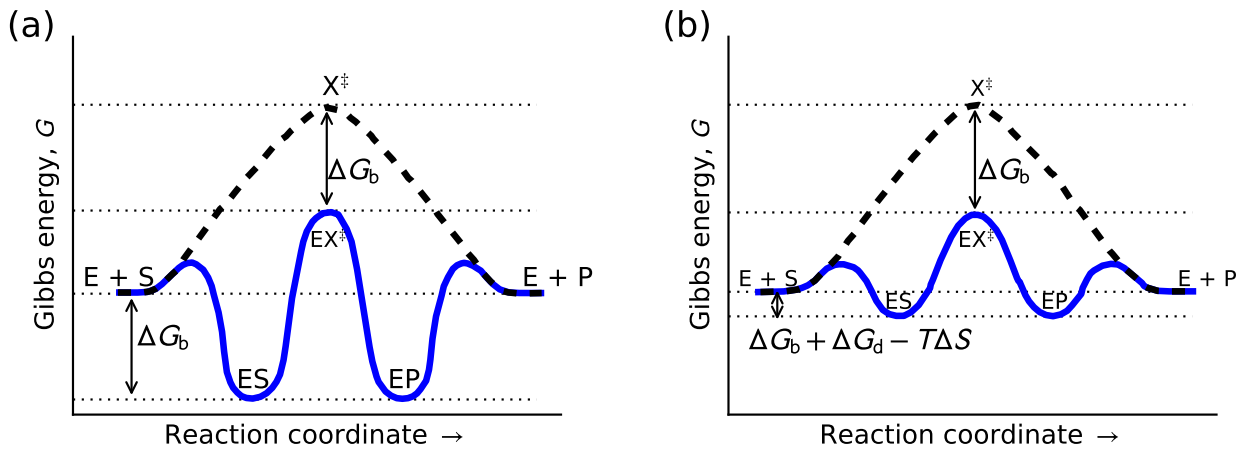

Figure 22: *Destabilisation of the ES complex is required for catalysis.* (a) When substrate and transition state are stabilised to the same extent, the activation energy from S to X‡ is the same as that from ES to EX‡. No destabilisation therefore means no catalysis. (b) When the ES complex is destabilised, the activation energy from ES to EX‡ is less than that from S to X‡. Therefore, destabilisation facilitates catalysis.

Because of the strong binding of enzymes to transition states, they can be inhibited by molecules that mimic the chemical structure of the transition state but are stable molecules (a normal transition state in an enzyme mechanism only exists for $10^{-14} - 10^{-13}$ s). Such molecules are called *transition state analogues*. While they do not bind as tightly to the enzyme as the true transition state, they nevertheless bind much tighter than the natural substrates (their K_s values can be from 10^3 up to $10^8\times$ lower than those of the substrate). Transition state-analogues also often form the basis of the development of new medicinal drugs where enzyme inhibitors are needed to treat diseases.

7.1.3 Proximity effect

The final mechanism of rate acceleration that will be discussed here is the proximity effect. This has to do with the correct positioning of substrate molecules with respect to each other. Binding to the enzyme reduces the degrees of freedom of movement, and thus leads to a loss of entropy (greater order). This can increase the rate of a reaction by around $10^4\times$.

Consider a bimolecular reaction $\mathrm{A} + \mathrm{B} \rightarrow \mathrm{P}$ with a rate constant k_2 (the units are $\mathrm{M}^{-1}\ \mathrm{s}^{-1}$). The rate will then be given by $v = k_2 \times a \times b$. In contrast, for a unimolecular reaction $\mathrm{A} \cdot \mathrm{B} \rightarrow \mathrm{P}$ where the reactants occur in a pre-formed complex, the rate will be given by $v = k_1 \times (a \cdot b)$ with rate constant k_1 (the units are now s^{-1}).

The rate acceleration brought about by the proximity effect can be evaluated by calculating the *effective molarity* (EM). This quantity is defined as the ratio of the unimolecular to the bimolecular rate constants and has units of M. For the example in Fig. 23,

$$\mathrm{EM} = \frac{k_1}{k_2} \tag{90}$$

$$= \frac{5 \times 10^7\ \mathrm{s}^{-1}}{10\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}} \tag{91}$$

$$= 5 \times 10^6\ \mathrm{M} \tag{92}$$

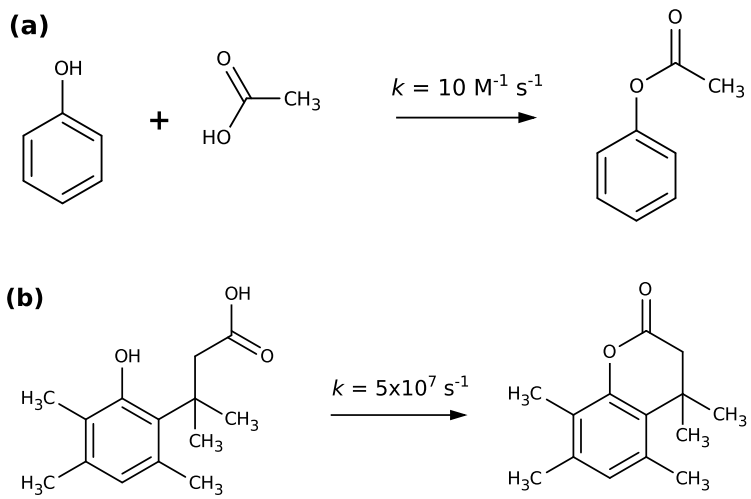

Figure 23: *Illustrating the proximity effect.* Although the chemistry is exactly the same, the unimolecular reaction in (b) proceeds faster than the bimolecular reaction in (a) because the reagents are pre-positioned and held in place close to where they are reacting. In (a), each reactant can diffuse away.

The EM can be interpreted as the 'effective concentration' of the second chemical group on the pre-complexed substrate. Consider the carboxyl group (-COOH) on the substrate in Fig. 23(b). Assume the substrate is present at a concentration of 1 M. Assume now that the first substrate in Fig. 23(a) (phenol) is also present at 1 M. For the reaction in (a) to proceed at the same rate as that in (b), the second substrate (acetic acid) has to be present at a concentration of the EM, i.e. 5×10^6 M. Put differently, the -COOH group in (b) behaves as if it were present at a concentration of 5×10^6 M in free solution—hence the name effective molarity.

This section has dealt with the energetics of enzyme binding and catalysis. We have shown how enzymes catalyse chemical reactions to increase their rates by many orders of magnitude. The following sections deal with deriving mathematical expressions that describe how fast enzyme-catalysed reactions will proceed as a function of the concentrations of their substrates, products and inhibitors.

7.2 The reversible Michaelis-Menten equation

In the Biochemistry 214 course you were introduced to the Michaelis-Menten equation as a description of the kinetics of enzyme-catalysed reactions. *Revise your notes on this subject!*:

$$v = \frac{Vs}{K_m + s} \tag{93}$$

This form of the Michaelis-Menten equation has the serious shortcoming that it only considers irreversible reactions. This is fine for probing the mechanism of enzyme reactions in the test tube, but we are interested in the behaviour of enzymes in the cell where, in principle, all reactions are reversible. In this section we derive how incorporating reversibility changes the form of the Michaelis-Menten equation. We will see that the *reversible* Michaelis-Menten equation is a much more useful tool for describing the kinetic behaviour of reactions in living cells than the irreversible equation, which ignores the product.

Consider the following generalised reaction scheme for the enzyme-catalysed conversion from S to P:

$$\mathrm{E} + \mathrm{S} \underset{K_s}{\rightleftharpoons} \mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{EP} \underset{K_p}{\rightleftharpoons} \mathrm{E} + \mathrm{P} \tag{94}$$

Remember that the Michaelis-Menten treatment assumes that the two binding equilibria above (with dissociation constants K_s and K_p) are rapid when compared to the interconversion of ES and EP (defined by the rate constants k_2 and k_{-2}). Thus, substrate S is in equilibrium with enzyme-substrate complex ES, and product P is in equilibrium with enzyme-product complex EP.

If S is reacting in the initial absence of P, the concentration of EP is effectively zero. The forward rate reaches its limiting value, V_f, when the catalytic sites of the enzyme are saturated with S so that es may be assumed to be equal to the total enzyme concentration e_0.

$$V_f = k_2 es = k_2 e_0 \tag{95}$$

Similarly, if P is reacting in the initial absence of S, the concentration of ES is effectively zero. Here the reverse rate reaches its limiting value, V_r, when the catalytic sites of the enzyme are saturated with P so that ep may be assumed to be equal to e_0.

$$V_r = k_{-2} ep = k_{-2} e_0 \tag{96}$$

However, if both S and P are present, neither can completely saturate the enzyme because they compete with each other for binding to enzyme. For any given concentration of S, the fraction of S bound to the enzyme is reduced by increasing concentrations of P. For any given concentration of P, the fraction of P bound to the enzyme is reduced by increasing concentrations of S.

This is a *kinetic inhibition* of the forward and reverse rates of reaction. It is an additional and quite different effect from the normal thermodynamic mass-action effect experienced by all reactions (catalysed or not) that when they approach equilibrium their net rate decreases because of the increasing rate of the reverse reaction (as shown in the term $1 - \Gamma/K_{eq}$ above in Eq. 61).

With this background one can construct the reversible Michaelis-Menten rate equation.

1. We write the conservation equation for enzyme forms. The enzyme can exist either as free enzyme E, or bound to substrate (ES) or product (EP). The total enzyme concentration e_0 is thus the sum of all the different forms of E:

$$e_0 = e_f + es + ep \tag{97}$$

   where e_f denotes the concentration of free enzyme E, bound neither to S nor to P. As throughout this course, we use lowercase italics to denote concentrations.

2. The dissociation equilibria of S and P from the enzyme are characterised by the dissociation constants K_s and K_p respectively:

$$K_s = \frac{e_f \cdot s}{es} \qquad \text{and} \qquad K_p = \frac{e_f \cdot p}{ep} \tag{98}$$

   In principle, s and p above refer to the *free* concentrations of S and P respectively. However, most of the time substrate and product concentrations are *much larger* than enzyme concentrations, so that the fraction of S or P that is bound to the enzyme (in the form of ES or EP)

is negligible. For practical purposes, one can therefore *equate the total* substrate or product concentration (s_0 or p_0) *with the free* concentration (s_f or p_f), and we simply write s or p.

Using the above expressions for K_s and K_p, we rearrange the conservation equation (Eq. 97) into an expression for $Y_{\text{ES}} = es/e_0$, i.e. the fractional saturation of the enzyme with S:

$$\begin{aligned} Y_{\text{ES}} = \frac{es}{e_0} &= \frac{es}{e_f + es + ep} \\ &= \frac{\frac{e_f s}{K_s}}{e_f + \frac{e_f s}{K_s} + \frac{e_f p}{K_p}} \qquad \text{(from Eq. 98)} \\ &= \frac{\frac{s}{K_s}}{1 + \frac{s}{K_s} + \frac{p}{K_p}} \end{aligned} \tag{99}$$

Using the same approach, one can derive the expression for $Y_{\text{EP}} = ep/e_0$, i.e. the fractional saturation of the enzyme with P:

$$Y_{\text{EP}} = \frac{ep}{e_0} = \frac{\frac{p}{K_p}}{1 + \frac{s}{K_s} + \frac{p}{K_p}} \tag{100}$$

3. The net rate of the catalysed reaction is given by

$$v = k_2 es - k_{-2} ep$$

Substituting es and ep from Eqs. 99 and 100,

$$\begin{aligned} v &= k_2 e_0 \frac{\frac{s}{K_s}}{1 + \frac{s}{K_s} + \frac{p}{K_p}} - k_{-2} e_0 \frac{\frac{p}{K_p}}{1 + \frac{s}{K_s} + \frac{p}{K_p}} \\ &= \frac{k_2 e_0 \frac{s}{K_s} - k_{-2} e_0 \frac{p}{K_p}}{1 + \frac{s}{K_s} + \frac{p}{K_p}} \end{aligned} \tag{101}$$

Using the definitions of V_f and V_r (Eqs. 95 and 96), we obtain the **reversible Michaelis-Menten equation**:

$$\boxed{v = \frac{V_f \frac{s}{K_s} - V_r \frac{p}{K_p}}{1 + \frac{s}{K_s} + \frac{p}{K_p}}} \tag{102}$$

7.2.1 The Haldane relationship

Recall that the net rate of any chemical reaction (and thus also of an enzyme-catalysed reaction) at equilibrium is zero. Assume that the reaction above is in equilibrium. From Eq. 102 above it follows that:

$$\begin{aligned} V_f \frac{(s)_{eq}}{K_s} &= V_r \frac{(p)_{eq}}{K_p} \\ \text{Hence,} \quad \frac{(p)_{eq}}{(s)_{eq}} &= K_{eq} = \frac{V_f K_p}{V_r K_s} \end{aligned} \tag{103}$$

As before, $(s)_{eq}$ and $(p)_{eq}$ denote the *equilibrium concentrations* of S and P. Eq. 103 is known as the *Haldane relationship*, named after JBS Haldane, the scientist who first described it. The Haldane relationship relates the kinetic constants of the reversible Michaelis-Menten equation to the equilibrium constant. Because the equilibrium constant for a reaction *only depends on substrate and product concentrations* (and not on the enzyme; a catalyst does not alter the equilibrium), the Haldane relationship further shows that the four kinetic constants V_f, V_r, K_s and K_p are *not independent*. If three of them are known, the fourth can be calculated from their values and from the equilibrium constant.

Using the Haldane relationship, we can rewrite the reversible Michaelis-Menten equation:

$$\begin{aligned} v &= \frac{V_f \frac{s}{K_s} - V_f \frac{s}{K_s} \frac{p/s}{K_{eq}}}{1 + \frac{s}{K_s} + \frac{p}{K_p}} \qquad \text{(substitute } V_r \text{ and } \times \tfrac{s}{s}\text{)} \\ &= \frac{V_f \frac{s}{K_s} \left(1 - \frac{\Gamma}{K_{eq}}\right)}{1 + \frac{s}{K_s} + \frac{p}{K_p}} \end{aligned}$$

$$\boxed{v = V_f \times \frac{\frac{s}{K_s}}{1 + \frac{s}{K_s} + \frac{p}{K_p}} \times \left(1 - \frac{\Gamma}{K_{eq}}\right)} \tag{104}$$

Eq. 104 illustrates the important point that any rate equation can be written in the following form (see also Section 5.6):

$$v = V_f \times f_{kin} \times \left(1 - \frac{\Gamma}{K_{eq}}\right)$$

Here, V_f describes the maximum "rate capacity" of the reaction. $f_{kin} = \frac{s}{K_s}/(1 + \frac{s}{K_s} + \frac{p}{K_p})$ is a term describing the effect of saturation binding of S and P to the enzyme, and $(1 - \Gamma/K_{eq})$ is the thermodynamic (energetic) term which depends only on the concentration of substrates and products (compare Eq. 61).

Because Eq. 104 makes the equilibrium constant explicit, it *separates kinetic and thermodynamic aspects*. This is not true of the form of the equation given in Eq. 102.

7.3 Uncompetitive inhibition

As pointed out in the previous section, the product P acts as a competitive inhibitor for the substrate S in an enzyme following reversible Michaelis-Menten kinetics. The competitive inhibition is due to S and P binding at the same active site of the enzyme: binding of P blocks the active site and prevents S from binding. The extent of inhibition is quantified by the p/K_p term in the denominator of the rate equation (Eqs. 102 and 104), and is part of the kinetic term f_{kin}.

Sometimes, however, a product can also inhibit an enzyme *uncompetitively* by binding to the ES-complex, causing both S and P to be bound to the enzyme concomitantly and preventing catalysis. In this section, we investigate how uncompetitive inhibition will affect the rate equation for the reaction.

Consider the following reaction scheme:

$$\begin{array}{ccccccc} \mathrm{E+S} & \underset{K_s}{\rightleftharpoons} & \mathrm{ES} & \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} & \mathrm{EP} & \underset{K_p}{\rightleftharpoons} & \mathrm{E+P} \\ & & +\mathrm{P} & & & & \\ & K_{iu} & \downharpoonleft\uparrow & & & & \\ & & \mathrm{ESP} & & & & \end{array} \tag{105}$$

Because the enzyme can now exist in an additional form, ESP, we have to include this in the conservation equation for the different enzyme forms:

$$e_0 = e_f + es + ep + esp \tag{106}$$

The two binding equilibria for S and P to the *free* enzyme are the same as before (Eq. 98). The additional binding equilibrium for P to ES is characterised by a dissociation constant K_{iu} (K_i stands for *inhibition constant,* and the u stands for *uncompetitive*):

$$K_{iu} = \frac{es \cdot p}{esp} \tag{107}$$

We solve the above equation for esp, using the definition of K_s in Eq. 98:

$$esp = \frac{es \cdot p}{K_{iu}} = \frac{e \cdot s \cdot p}{K_s \cdot K_{iu}} \tag{108}$$

As previously, we rearrange the conservation equation (Eq. 106) into expressions for the fractional saturation of the enzyme with S, P, and both S and P:

$$Y_{\text{ES}} = \frac{es}{e_0} = \frac{\frac{s}{K_s}}{1 + \frac{s}{K_s} + \frac{p}{K_p} + \frac{s \cdot p}{K_s K_{iu}}} \tag{109}$$

$$Y_{\text{EP}} = \frac{ep}{e_0} = \frac{\frac{p}{K_p}}{1 + \frac{s}{K_s} + \frac{p}{K_p} + \frac{s \cdot p}{K_s K_{iu}}} \tag{110}$$

$$Y_{\text{ESP}} = \frac{esp}{e_0} = \frac{\frac{s \cdot p}{K_s K_{iu}}}{1 + \frac{s}{K_s} + \frac{p}{K_p} + \frac{s \cdot p}{K_s K_{iu}}} \tag{111}$$

The net rate of the catalysed reaction is again given by

$$v = k_2 es - k_{-2} ep$$

As before, we substitute es and ep from Eqs. 109 and 110,

$$v = \frac{k_2 e_0 \frac{s}{K_s} - k_{-2} e_0 \frac{p}{K_p}}{1 + \frac{s}{K_s} + \frac{p}{K_p} + \frac{s \cdot p}{K_s K_{iu}}} \tag{112}$$

Using the same definitions of V_f and V_r as before (Eqs. 95 and 96), and factorising the denominator, we obtain the **reversible Michaelis-Menten equation with uncompetitive product inhibition**:

$$\boxed{v = \frac{V_f \frac{s}{K_s} - V_r \frac{p}{K_p}}{1 + \frac{s}{K_s}\left(1 + \frac{p}{K_{iu}}\right) + \frac{p}{K_p}}} \tag{113}$$

or, in the form separating the thermodynamic and kinetic terms:

$$\boxed{v = \frac{V_f \frac{s}{K_s}\left(1 - \frac{\Gamma}{K_{eq}}\right)}{1 + \frac{s}{K_s}\left(1 + \frac{p}{K_{iu}}\right) + \frac{p}{K_p}}} \tag{114}$$

The additional term $\left(1 + \frac{p}{K_{iu}}\right)$ in the denominator of Eqs. 113 and 114 quantifies the extent of uncompetitive inhibition by P. The above equations also show that when $K_{iu} = \infty$ (P does not bind to ES), the inhibition is *purely competitive* (given by the term p/K_p). Likewise, if $K_p = \infty$ (P does not bind to the free enzyme), the inhibition is *purely uncompetitive* (given by the term $1 + \frac{p}{K_{iu}}$). In general, neither K_p nor K_{iu} is infinitely large, so that the inhibition by P will have a competitive and an uncompetitive component (this is called *mixed inhibition*).

7.4 Cooperativity and the reversible Hill equation

The reversible Michaelis-Menten equation discussed above is a vast improvement on the commonly used irreversible form, because it successfully takes into account the reversibility of enzyme-catalysed reactions and separates the thermodynamic and kinetic contributions to reaction rate. However, it fails to explain the following phenomena:

- Many enzymes follow *cooperative kinetics.* These enzymes have multiple binding sites for substrates, and the binding of one substrate molecule changes the affinity of the other binding sites so that subsequent substrate molecules bind better (positive cooperativity) or worse (negative cooperativity). Positive cooperativity leads to a *sigmoidal* dependence of reaction rate on substrate concentration, whereas the v vs. s curve is *flattened* for an enzyme displaying negative cooperativity (see Biochemistry 214). The reversible Michaelis-Menten equation cannot explain cooperativity.

- Many enzymes in biosynthetic metabolic pathways are subject to allosteric *feedback inhibition*, where an end-product further down in the metabolic pathway inhibits the committing enzyme by binding to a separate (allosteric) site, which is distinct from the active site. Such feedback inhibition usually also follows sigmoidal kinetics, and can likewise not be explained by the reversible Michaelis-Menten equation.

At the beginning of this course we set ourselves the major goal to come to a new view of metabolic regulation. A very common case where this becomes important is a biosynthetic pathway with allosteric feedback, which is discussed below (Fig. 39). To really understand how these allosteric enzymes behave in a metabolic pathway, we need to understand their kinetics. This is what this section is about.

7.4.1 The reversible Hill equation

Consider the scheme in Fig. 24. It shows a kinetic model for a two-site enzyme. Each active site can either bind a molecule of S or a molecule of P. The enzyme can also exist in states where one or both sites are empty.

The arrows show all the possible transitions between the different enzyme states. The horizontal arrows represent catalytic conversions between S and P; the forward rate constant for the conversion from S to P is k_f, and the reverse constant (P to S) is k_r. The arrows linking the vertical levels represent binding/dissociation equilibria for S or P.

In the bottom level of Fig. 24 the rate constants k_f and k_r are multiplied by 2 because there are two molecules of S bound to the enzyme in the ES_2 species (and two molecules of P in EP_2), whereas there is only one S or P in each of ES, EP or ESP. This makes the likelihood S or P reacting in the ES_2 or EP_2 species twice as high as in the other species (because any one of the two molecules can react).

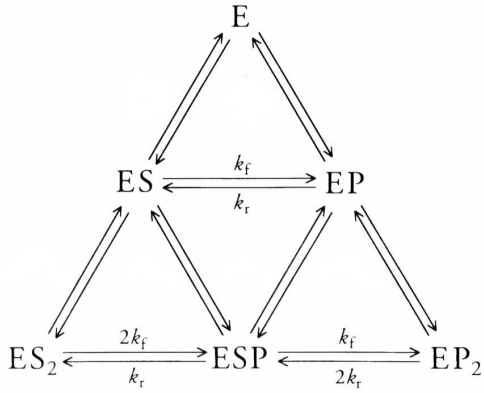

Figure 24: *Kinetic model for a two-site enzyme.*

The 2 thus is a "statistical factor" to take into account that two S molecules or two P molecules are bound to the enzyme at the same time.

In an analogous manner, by taking all the horizontal arrows into account, the net rate v of conversion from S to P can be given by:

$$v = k_f(es + 2es_2 + esp) - k_r(ep + 2ep_2 + esp) \tag{115}$$

Note how the concentrations es_2 and ep_2 are multiplied by 2 because they have two molecules of S or P bound. The term esp occurs in both the forward and the reverse reaction rates, because the species ESP can react to form either EP_2 (forward reaction) or ES_2 (reverse reaction).

Eq. 115 can be solved for v (we will not give the derivation here):

$$v = \frac{V_f \frac{s}{s_{0.5}} \left(1 - \frac{\Gamma}{K_{eq}}\right) \left(\frac{s}{s_{0.5}} + \frac{p}{p_{0.5}}\right)}{1 + \left(\frac{s}{s_{0.5}} + \frac{p}{p_{0.5}}\right)^2} \tag{116}$$

where $s_{0.5}$ is the concentration of S giving half-maximal forward reaction rate in the absence of P, and $p_{0.5}$ is the concentration of P giving half-maximal reverse reaction rate in the absence of S (note that we cannot use K_s and K_p here, because the experimentally determined $s_{0.5}$ and $p_{0.5}$ are not dissociation constants). V_f, Γ and K_{eq} are defined as previously.

We now introduce the following shorthand: $\sigma = s/s_{0.5}$ and $\pi = p/p_{0.5}$. We use this to rewrite the above equation in a simpler form:

$$v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K_{eq}}\right) (\sigma + \pi)}{1 + (\sigma + \pi)^2} \tag{117}$$

Eq. 117 is the **reversible Hill equation** for an enzyme with two active sites. The **general form** reads

$$\boxed{v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K_{eq}}\right) (\sigma + \pi)^{h-1}}{1 + (\sigma + \pi)^h}} \tag{118}$$

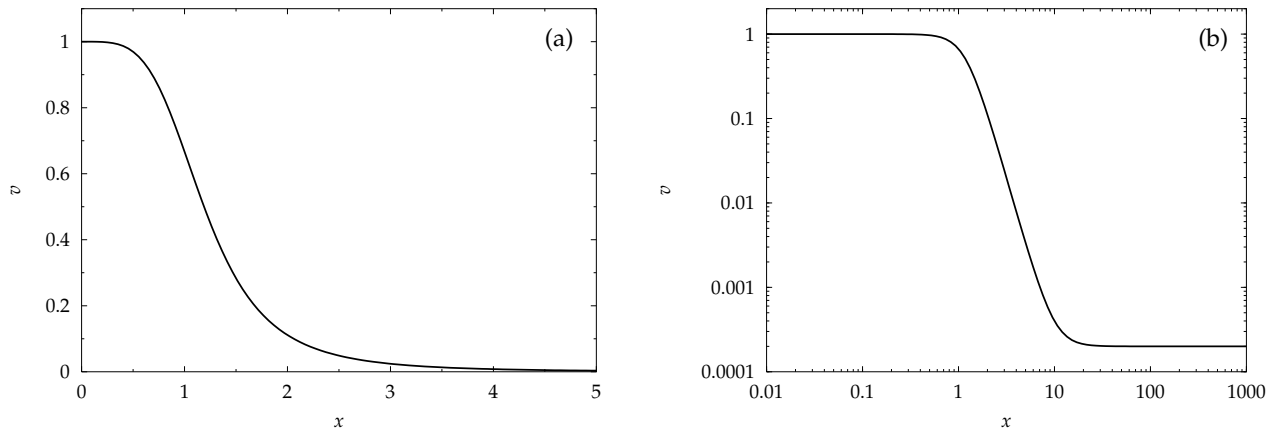

Figure 25: *Graphs of the reversible Hill equation with modifier.* (a) linear coordinates; (b) logarithmic coordinates. The graphs show the dependence of reaction rate v on modifier concentration x. The fixed parameters are: $V_f = 2$, $s = s_{0.5} = 1$, $p = 1$, $p_{0.5} = 10^4$, $K_{eq} = 10^4$, $h = 4$, $\alpha = 10^{-4}$, and $x_{0.5} = 1$.

Self-study exercises

1. The parameter h is known as the Hill coefficient and can vary between 1 and the number of active sites on the enzyme. The greater h, the stronger is the cooperativity of the enzyme. What happens if $h = 1$?

2. How does Eq. 118 reduce when $p = 0$? (This reduced equation is known as the *irreversible* Hill equation and was first described in 1910. It is widely used in biochemistry to describe cooperative enzymes.) Incidentally, the reversible Hill equation was developed by Prof Hofmeyr from our department in 1997.

3. How does Eq. 118 reduce when $s = 0$? Why is the reaction rate negative?

4. How many reasons can you think of why the reversible Hill equation is better than the irreversible one?

7.4.2 Modifier effects in the reversible Hill equation

Although the reversible Hill equation quantitatively describes cooperativity and gives a sigmoidal rate vs. substrate concentration graph, Eq. 118 in the form given above cannot account for modifier effects, i.e. inhibition or activation by an allosteric modulator. However, these allosteric modifier effects are central to understanding metabolic regulation because of the ubiquitous feedback loops present throughout metabolism. Fortunately there is a way to extend the reversible Hill equation to incorporate a modifier.

Assume that X is a modifier binding to the enzyme, x is its concentration and $x_{0.5}$ the concentration at which X exerts half of its total effect. Let $\xi = x/x_{0.5}$. The **reversible Hill equation with modifier** then reads:

$$\boxed{v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K_{eq}}\right) (\sigma + \pi)^{h-1}}{(\sigma + \pi)^h + \frac{1+\xi^h}{1+\alpha\xi^h}}} \tag{119}$$

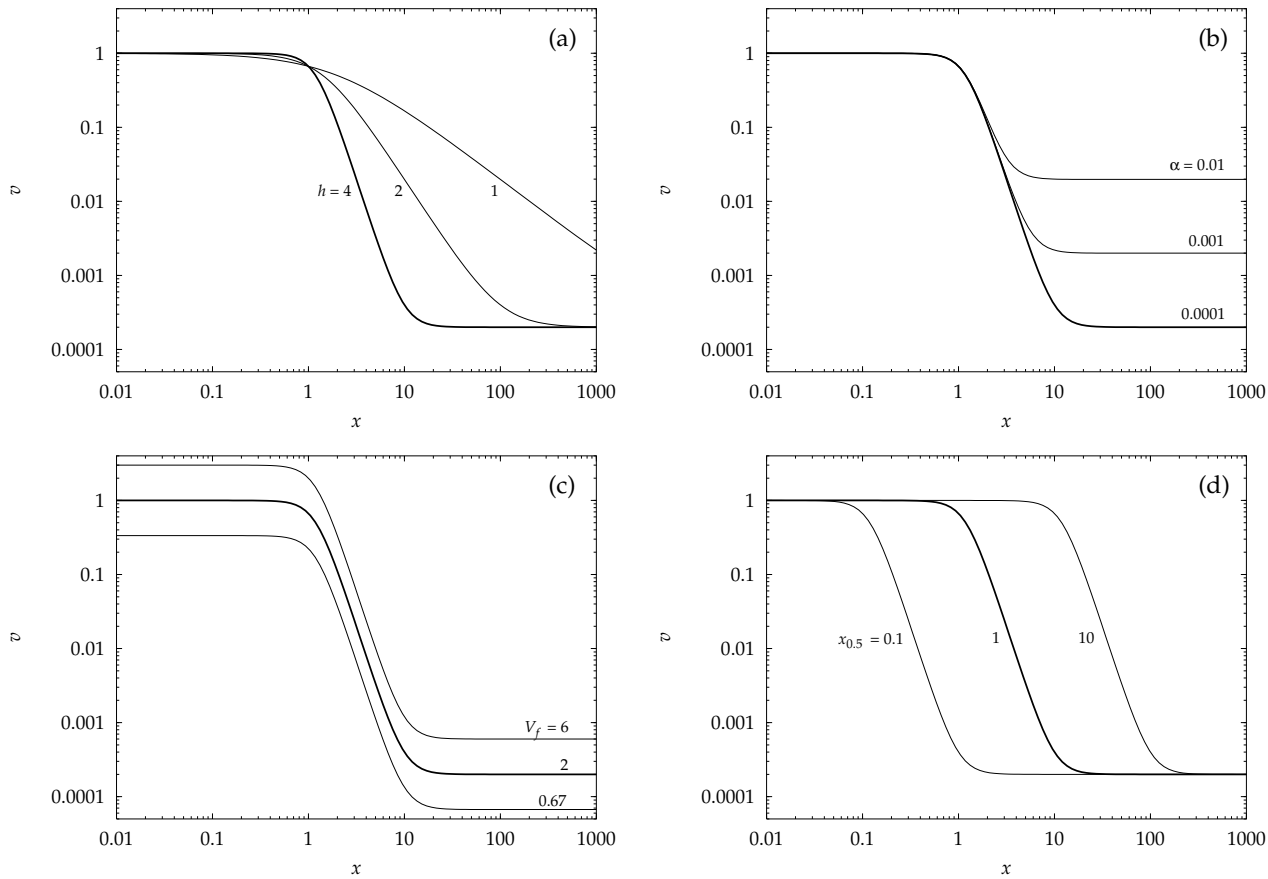

Figure 26: *The effect of changing the parameters in the reversible Hill equation.* The reference parameters were as in Fig. 25 and are indicated by the bold line in each graph. In each case, one of the parameters is varied around its reference value while keeping the others constant at their reference values. The parameters varied are (a) h; (b) α; (c) V_f; and (d) $x_{0.5}$.

Eq. 119 applies equally well to allosteric inhibition and activation. Check this for yourself: if $\alpha < 1$, then X is an inhibitor; if $\alpha > 1$, then X is an activator; if $\alpha = 1$, then X has no effect on the reaction rate.

Fig. 25 shows graphically how the reversible Hill equation (Eq. 119 above) depends on the concentration of the modifier X, while keeping the other concentrations and parameters constant. We plot the rate as a function of x here, because the modifier plays the role of allosteric inhibitor in many metabolic pathways. We will return to the the shape of the graphs in Fig. 25 once we discuss supply-demand analysis in Section 11.3.

Fig. 25 (a) shows v vs. x in linear space, whereas Fig. 25 (b) shows the same graph in double-logarithmic space. We show the logarithmic graph, because you can directly read off from the graph the reaction order with respect to X (see Section 5.2.1). The importance of rate characteristics in logarithmic space will be further discussed in detail in Section 9.2.

For now, it is sufficient to note that both graphs in Fig. 25 show a sigmoidal dependence of v on x, and X acts as an inhibitor of the enzyme (v decreases as x increases). The dependence of v on s (the substrate concentration) is also sigmoidal, but in that case v *increases* with increasing s (this graph is not shown here).

Meaning of parameters You might be wondering what is the effect of changing the parameters in the reversible Hill equation, and how this affects the shape of the graph.

Changing the value of the hill coefficient h changes the degree of cooperativity in the binding of X (Fig. 26(a)). The lower h, the less steep is the sigmoidal curve and the weaker is the cooperativity. When $h = 1$, there is no cooperativity and the curve is no longer sigmoidal.

Changing the value of α varies the extent to which X inhibits the enzyme (Fig. 26(b)). If we lower α, the plateau of maximum inhibition shifts down and the enzyme is inhibited more. If $\alpha = 1$, there is no effect of X on the reaction rate, and if $\alpha > 1$, X activates the enzyme (the latter two cases are not shown on the graph).

Changing the V_f of the enzyme varies its total capacity. As a consequence, the whole curve shifts up when V_f is increased and down when V_f is decreased (Fig. 26(c)).

Finally, changing $x_{0.5}$ varies the concentration at which X is an effective inhibitor (Fig. 26(d)). As a consequence, the curve shifts to the left if $x_{0.5}$ is decreased and to the right if $x_{0.5}$ is increased. Observe from the graph that the enzyme is halfway inhibited ($v = 0.5$) at a point where x is approximately equal to $x_{0.5}$.

In Section 11.3 we will see how all the different parameters in the reversible Hill equation work together to ensure effective regulation in a supply-demand system. But, before we can do that we need to see what happens to the behaviour of a network of reactions when we couple them together. In the cell, thousands of coupled reactions all operate at the same time. However, to understand the concepts we will start with the simplest possible system consisting of two coupled reactions.

8 Coupled reactions and rate characteristics

When faced with the metabolic map we are struck by its seemingly bewildering complexity. Just as we search for structural motifs to try to make sense of protein structure, so should we look for underlying structure in metabolic networks. When studying the dynamic behaviour of metabolic networks, we are not so much interested in reaction chemistry and mechanism (the main concern of metabolic studies thus far), but rather in the different ways in which reactions are coupled. We first show that the metabolic network is built up from a few basic linkage types and structures. This provides a good background against which a kinetic model of metabolism can be developed. We are of course primarily interested in metabolic structures in which reactions are catalysed by enzymes, but it should be emphasised that the kinetic model we develop here is applicable to any system of coupled chemical reactions. Although coupled reaction networks show many of the properties of electrical and hydrodynamic networks, there is one property of reaction networks that makes them unique, richer in behaviour, and therefore more interesting—this property is stoichiometry, the fixed ratios in which molecules react with each other. The existence of stoichiometry complicates the study of network behaviour, but this is more than compensated for by the new possibilities it opens up for novel behaviour. Although stoichiometry will of course be incorporated into the kinetic model right from the start, we will not study it systematically in this course.

8.1 The basic linkages and structures in metabolism

A fundamental feature of metabolic pathways is that any two functional steps, usually enzyme-catalysed reactions or transport steps, are linked by metabolites common to both (the product of one step becomes the substrate of the next). The two different types of linkages are shown in Fig. 27.

An important difference between these two types of linkage is that a type 1 linking metabolite is part of the material being metabolised and is passed on, whereas a type 2 linking metabolite alternates between two or more forms of a chemical group (called a **moiety**); it forms a permanent part of the system. The concentration of type 1 linker is in theory free to assume any value (however, there may be physical constraints on the concentrations of metabolic intermediates due to limits in the solvent capacity of water). On the other hand, the total concentration of the different forms of a type 2 linking metabolite is constant under the condition that the *de novo* synthesis and degradation of the moiety be slow in comparison to the interconversion of the different forms. Such type 2 structures are called **moiety-conserved cycles**. The concept of moiety conservation is so important that Reich and Sel'kov described energy metabolism as "open flow through networks of moiety-conserved cycles".

In metabolism there is a tendency to form chains consisting of only one type of linkage (Fig. 28). Many of the familiar metabolic pathways have as backbone a chain of type 1 linkages. On the other hand, in the electron transfer chain all linkages are of the second type (each carrier existing in two forms, the total amount being conserved). Often a type 2 chain might start from one of the steps of a type 1 chain and perhaps terminate in another type 1 chain or even in a later step of the same type 1 chain.

8.2 The kinetic behaviour of the basic linkage types

We start off our exploration of the kinetic behaviour of metabolic structures by investigating the properties of simple reaction systems connected by the first basic linkage type. We shall find that

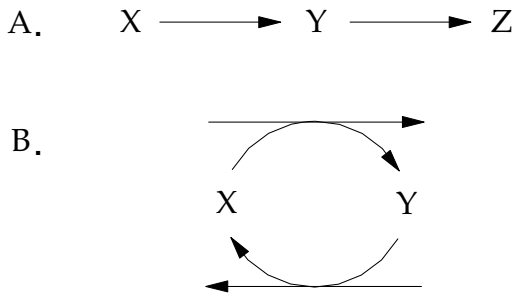

Figure 27: *The two ways in which enzymic reactions are linked.* A. The second enzyme takes the linking metabolite, Y, on to form a new substance, Z. B. The second enzyme reverses the action of the first enzyme as far as the linking metabolite is concerned. For example, if the first enzyme oxidises X, the second reduces it, or if the first enzyme phosphorylates X to form Y, the second dephosphorylates Y back to X. Of course, the second reaction is not a simple reversal of the first, but a different reaction catalysed by a different enzyme (e.g., the first enzyme may be a kinase that uses ATP to phosphorylate X, in which case the second enzyme usually is a phosphatase that hydrolyses the phosphoester bond). This is the well-known metabolic feature of reactions that are coupled by the interconversion of different forms of cofactors, such as NAD^+/NADH or ATP/ADP. These reactions are usually bimolecular reactions in which some or other chemical group (such as a hydride or phosphoryl group in the above examples) is transferred from a donor to an acceptor compound.

most of the features pertinent to an understanding of metabolic behaviour, control and regulation are inherent in these simple systems.

8.2.1 Linkage Type 1: Two coupled irreversible reactions

The simplest example of two reactions linked by type 1 is given in Fig. 29. In spite of the fact that the irreversible kinetics assumed in this first example of this system have no metabolic significance, we shall devote what will seem an inordinate amount of time and space to it; we ask your forbearance: the concepts illustrated are of far greater importance than the kinetic details of the system.

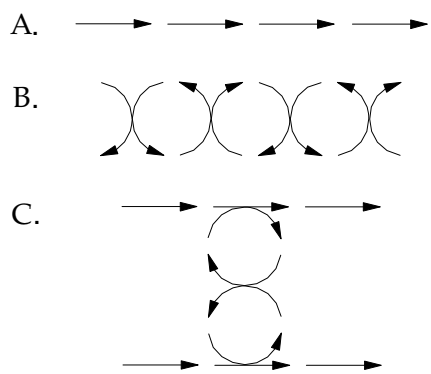

Figure 28: *Chains of reactions consisting of:* A. type 1 linkages, B. type 2 linkages, C. type 2 linkages starting from and ending in a type 1 chain.

$$\mathrm{S} \xrightarrow{v_1} \mathrm{X} \xrightarrow{v_2} \mathrm{P}$$

Figure 29: *Two irreversible reactions connected by a type 1 linkage.*

8.2.2 Rate equations

If we assume that the reactions are uncatalysed and governed by simple mass-action kinetics, the rates at which each of the two irreversible reactions proceed are given by the following rate equations:

$$v_1 = k_1 s \tag{120}$$
$$v_2 = k_2 x \tag{121}$$

For clarity's sake, we use lowercase italic symbols to denote concentrations, omitting the usual square brackets. The constants k_1 and k_2 are *rate constants* with units of time^{-1}. Both reactions have a *reaction order* of one, as there is only one concentration term in each rate equation.

We will deal with kinetics and reaction order in greater detail later in Section 5. For the time being, we continue with this simple example.

8.2.3 Time-dependent behaviour of the system

If we start the system off with a set of initial conditions, say $s = 5$ mM, $x = p = 0$ mM, $k_1 = k_2 = 1$ min^{-1}, how do the rates and concentrations develop with time? This question is impossible to answer unless we specify a crucial property of the system, namely whether it is *closed* or *open*. If the system is **closed** it means that mass cannot enter or leave the system; because both reactions are irreversible, all the S molecules will eventually end up as P. For the closed system this ultimate stationary state is an *equilibrium* state where all net reaction rates are zero (as this system has no reversible reactions, this is not so obvious). If the system is **open** it means that, in this example, the pathway substrate S is replenished and the product P is removed by reactions outside the system. The system therefore interacts with its environment through the exchange of matter. We shall consider an important case of an open system where S and P are buffered at their initial concentrations and therefore remain constant. Note, however, that buffering of P is not important in this system in the sense that accumulation of P cannot affect the system in any way; reaction 2 is irreversible and its rate is therefore insensitive to changes in p.

The time course for changes in the concentrations S, X, and P is described by a set of **balance equations**, one for each compound. Each balance equation is the time derivative of a reactant concentration, which is equal to the sum of rates of that produce the reactant minus the sum of the rates that consume the reactant:

$$\frac{ds}{dt} = -v_1 = -k_1 s \tag{122}$$
$$\frac{dx}{dt} = v_1 - v_2 = k_1 s - k_2 x \tag{123}$$
$$\frac{dp}{dt} = v_2 = k_2 x \tag{124}$$

This set of equations comprise the **kinetic model** of the system. Although it is possible to obtain for this simple linear system an analytical solution for each of the concentrations as a function of time, we can also solve the system by numerical integration on computer using the initial conditions described above. For complex metabolic systems in general there are no analytical solutions; computer simulation and experimental analysis are the only avenues available.

Implicit in these balance equations is an important simplifying assumption: The reactants are at all times uniformly distributed through the space occupied by the compartment in which they occur, i.e., no concentration gradients exist within the system, except across membranes (this assumption has its counterpart in the chemical engineer's concept of a "well-stirred reactor"). Such reactants can be regarded as thermodynamically defined in the sense that they can be assigned a chemical potential. In large compartments and when diffusion processes are slow there may be a concentration gradient of a specific metabolite, which therefore cannot be represented by a uniform pool with a measurable size; here the balance equations must also contain diffusion terms.

For the *closed* system the concentration time-courses are given in Fig. 30A and the reaction rates as a function of time in Fig. 30B. The substrate S is consumed continuously, while P is produced continuously; the concentration x increases transiently, reaches a maximum where its rate of production balances its rate of consumption (compare the maximum in the time course of x in Fig. 30A with the point in Fig. 30B where the two rate curves cross), after which it decreases to zero. The rate of reaction 1 decreases as S falls to zero, while that of reaction 2 initially increases as X increases, then goes through a maximum where the production of X matches its consumption, and finally falls off to zero when all the S has been converted to P.

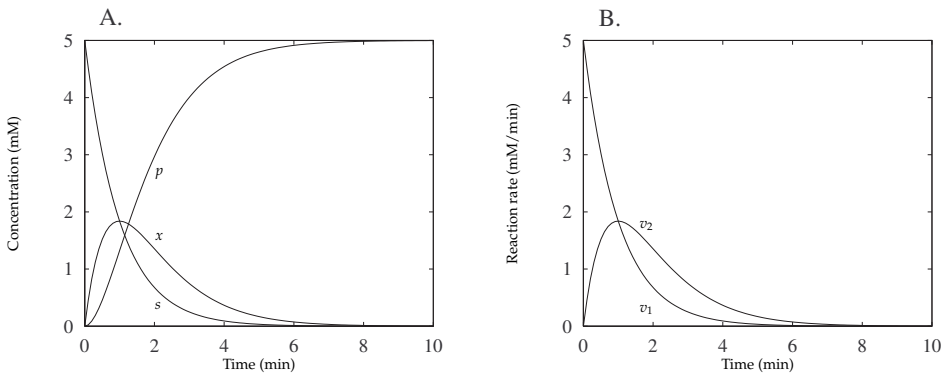

Figure 30: *Concentration (A) and rate (B) time-courses for the closed system in Fig. 29.* Simulation conditions were $s = 5$ mM, $x = p = 0$ mM, $k_1 = k_2 = 1$ min^{-1}. All concentrations were allowed to vary within the total mass conservation constraint of 5 mM.

For the *open* system where s and p are buffered at their initial concentration the picture is quite different (Figs. 31A and B). Here the system spontaneously evolves to a state in which the concentration of X remains constant in time, and in which there is a constant flux of matter through this pool of X; X is therefore produced and consumed at the same rate. This stationary state in which the variable concentrations remain constant in time but the reaction rates are non-zero is called the **steady state**. We therefore say that the system evolves from the initial state through a continuous series of **transient states** until it reaches the steady state. In terms of the balance equations the steady state is

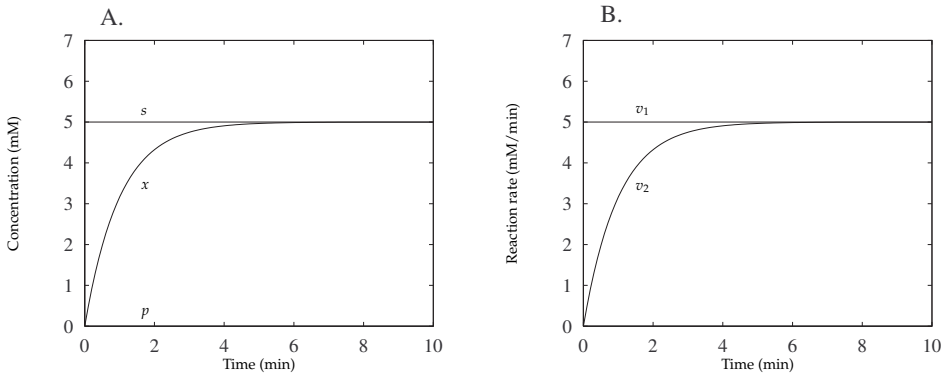

Figure 31: *Concentration (A) and rate (B) time-courses for the open system in Fig. 29.* Simulation conditions were: $s = 5$ mM, $x = p = 0$ mM, $k_1 = k_2 = 1$ min^{-1}. The concentrations of S and P were clamped at their initial values.

reached when the balance equations for the internal variable concentrations are zero:

$$\frac{dx}{dt} = \bar{v}_1 - \bar{v}_2 = k_1 s - k_2 \bar{x} = 0 \tag{125}$$

where the bar over x denotes the steady-state concentration and the bars over v_1 and v_2 steady-state rates. For this system in steady state it follows that

$$\bar{v}_1 = \bar{v}_2 = J \tag{126}$$

These steady-state rates are generally called *steady-state fluxes*, or just the **flux**. Flux is symbolised by J.

What is the steady-state concentration of X and the steady-state flux J? Although an analytical solution can here be easily obtained from eq. 125 as

$$\bar{x} = \frac{k_1 s}{k_2} \tag{127}$$

which, if inserted into either of the two rate equations 120 or 121, gives the expression for the flux,

$$J = k_1 s \tag{128}$$

a graphical solution on a graph known as a **rate characteristic** gives not only the steady-state solution, but also a picture of how changes in x around the steady state affects the rates through the two parts of the system. We use the fact that x is the only variable that could affect v_1 and v_2 (remember that in the open system s and p are constant). The functional dependence of v_1 and v_2 on x can be plotted on the same graph of rates versus x (Fig. 32). The value of x at which the rates are equal is the steady-state concentration; at that point the rate through the reactions equals the steady-state flux J. The v_1-characteristic shows that it is independent of x, while the v_2-characteristic show a linear dependence on x with slope k_2.

The steady state is completely *determined* by three factors: (i) the topology (network structure) of the system (the way in which the reactions are connected); (ii) the functional form of the rate

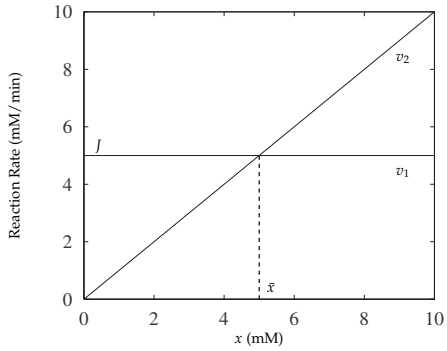

Figure 32: *Rate characteristics that show the dependence of individual reaction rates v_1 and v_2 of the system in Fig. 29 on the concentration of X.* The value of x at which the rates are equal is the steady-state concentration of X. The concentration of S is 5 mM; $k_1 = k_2 = 1$.

equations; (iii) the constant entities in the rate equations, in general called the **parameters** of the system (the parameters of this example are s, p, k_1, and k_2). In turn, the steady state is *characterised* by the steady-state values of the internal *variable* concentrations ($\bar{x}$) and fluxes (J).

A fundamental property of all metabolic systems is that they are open; therefore the steady state is a characteristic state of living systems. The equilibrium state in which all closed systems end up is of no interest to a study of living systems; systems in equilibrium are dead. By this we do not imply that the equilibrium constants of metabolic reactions are not important; to the contrary, they are crucial to any understanding of the driving forces of any chemical reaction. This will become clear when we consider reactions networks that contain reversible reactions. We now investigate a number of important properties of the steady state.

8.2.4 Properties of the steady state

Uniqueness In this system there is only one steady state possible for any specific set of parameters; the steady state is *unique*. This can clearly be seen from Fig. 32; changing the parameters will shift the steady state around, but qualitatively the graph will not change—only one steady-state point obtains (the lines for v_1 and v_2 intersect only once). This need not necessarily be so: for more complex (but metabolically quite realistic) systems the situation can arise where, for a specific set of parameters, more than one steady-state is possible; which one is attained depends on where the system starts off. Such systems are said to have *multiple steady states*, or are said to exhibit *multi-stationarity*.

Stability Fig. 32 shows another important property of this particular steady state—it is *dynamically stable* to fluctuations in the concentration of X. By that we mean that, if the steady state is perturbed by changing x, the same steady state is ultimately re-established. Again this is easy to see from Fig. 32 (which is turning out to be a very useful type of graph): imagine an increase in x away from the steady state; as $v_2 > v_1$, it follows that $dx/dt < 0$ so that the system pushes x back to the original steady state. The same holds for a decrease in x away from the steady state; $v_1 > v_2$ and $dx/dt > 0$, so that x increases to its original steady-state value. Actually, we can start off the system at any value of x; the same steady state will be approached and the system is therefore *globally stable*.

It is also possible to obtain a measure of how long it takes for the perturbed system to relax back to the steady state; this must depend on how rapidly the rate of change dx/dt, and therefore the difference $v_1 - v_2$, changes with x. For this system,

$$\frac{d(v_1 - v_2)}{dx} = -k_2 \tag{129}$$

The more negative this value, the faster the system returns to the steady state. The absolute value of the inverse of this quantity ($1/k_2$) is the relaxation half-time; if the steady-state concentration x is perturbed by a small quantity δx it takes $1/k_2$ time units to move back $0.5\delta x$ towards steady state.

In this section, we have examined the time-dependent behaviour of two coupled reactions following mass-action kinetics. We have seen that closed systems tend to equilibrium, whereas open systems tend to the steady state. Of course, simple mass-action kinetics are not very relevant for living systems, since cellular reactions are catalysed by enzymes and follow enzyme kinetics as has been discussed in Section 7. How this fact affects the behaviour of coupled reactions and their rate characteristics, is the topic of the next section. The section also introduces yet another tool into our toolbox, i.e. *metabolic control analysis*, which describes and quantifies how the steady state is controlled.

9 Metabolic control analysis of the steady state

9.1 Two coupled reactions catalysed by Michaelis-Menten enzymes

With our knowledge of energetics and enzyme kinetics, we now take a fresh look at rate characteristics. In Section 8, we only considered a system of two irreversible coupled reactions that obey mass-action kinetics (see Fig. 29). Of course, neither pure mass action kinetics nor irreversible reactions are relevant for living systems. Because the chemical reactions in living systems are catalysed by enzymes, their kinetics are more complex (e.g., reversible Michaelis-Menten or reversible Hill kinetics, discussed in Section 7). Here, we will briefly discuss a system of two coupled enzyme-catalysed reactions, as in Fig. 29, with the important addition that enzyme 1 now obeys reversible Michaelis-Menten kinetics and enzyme 2 obeys irreversible Michaelis-Menten kinetics. Schematically, this can be represented as follows:

$$\mathrm{S} \underset{}{\overset{v_1}{\rightleftharpoons}} \mathrm{X} \xrightarrow{v_2} \mathrm{P}$$

Figure 33: *A reversible reaction coupled to an irreversible reaction by a type 1 linkage.*

Fig. 34 shows the rate characteristics for this system, i.e. how the rates v_1 and v_2 vary with the concentration of the linking metabolite, x. The dotted lines on the figure show how the rates v_1 and v_2 would vary with x if the reactions obeyed simple mass-action kinetics. (Compare the dotted lines in Fig. 34A with Fig. 32; the only difference is that the rate v_1 now decreases with increasing x, whereas it remained constant in Fig. 32. This is because reaction 1 is now reversible and inhibited by the concentration of its product, x, whereas v_1 was irreversible in Fig. 32 and hence insensitive to x.)

In contrast to the linear dependence of v_1 and v_2 on x for mass-action kinetics, Fig. 34A now shows the familiar hyperbolic dependence of v_1 and v_2 on x for Michaelis-Menten reactions. v_1 decreases as x increases because X is the product of reaction 1 and this reaction is reversible. High concentrations of product will thus inhibit reaction 1 (see Section 7.2 on the reversible Michaelis-Menten equation). Reaction 2 is irreversible, and therefore the dependence of v_2 on x in Fig. 34A is hyperbolic as expected from the ordinary irreversible Michaelis-Menten equation.

Although the rate characteristics in Fig. 34A are non-linear, their intersection nevertheless gives the prevailing steady-state point and the values of J and $\bar{x}$ can be read off directly from the graph, as indicated on the figure. The steady state can in this case also clearly be seen to be dynamically stable: if x increases above the steady-state value, $v_2 > v_1$ so that $dx/dt < 0$ and the system pushes the value of x back to the steady state; likewise, if x decreases below the steady-state value, $v_2 < v_1$ so that $dx/dt > 0$ and the system again pushes the value of x back towards the steady state.

Fig. 34B shows the rate characteristics in double-logarithmic space. As explained below in Section 9.2, this graph shows the relative change (or % change) in v_1 and v_2 for a 1 % change in x, or put differently, how sensitive (or 'elastic') v_1 and v_2 are to changes in x. The figure shows that the saturation terms in the Michaelis-Menten equations affect their sensitivities towards x so that they do not correspond to the mass-action ones. This can be seen most clearly for v_2: whereas a doubling

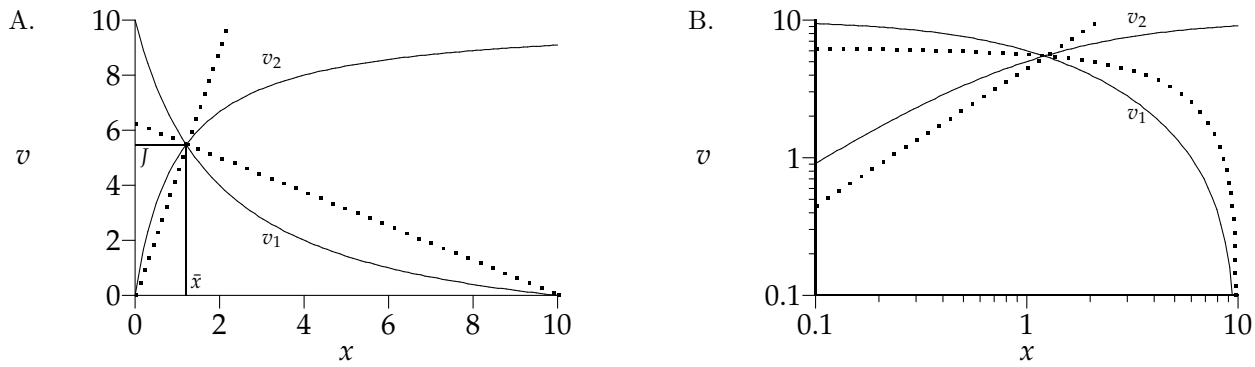

Figure 34: *Rate characteristics of the reactions in Fig. 33 with respect to the linking metabolite X.* A. Unscaled rate characteristics; B. Log-log form of the rate characteristics. The point of intersection is the steady state $(J, \bar{x})$. The solid lines were calculated from rate equations for a reversible Michaelis-Menten equation (v_1) and an irreversible Michaelis-Menten equation (v_2). The dotted lines were calculated from simple mass-action rate equations. The differences between the solid and the dotted curves express how the saturation terms in the kinetic equations of the catalysed system affect the behaviour of the reactions.

in x will always lead to a doubling in v_2 for the mass-action case (the slope of the dotted line equals 1), this does not hold true for the Michaelis-Menten case: here, doubling of x leads to doubling of v_2 at low values of x, but as x increases, v_2 becomes less and less sensitive towards x (because of the saturation of enzyme 2 with X).

9.2 Logarithmic scales and relative changes

The rate characteristic of Fig. 34B is plotted in double-logarithmic space. There is a good reason for this: on such a graph equal distances along the axes refer to equal relative (or fractional, or %) changes, whereas on the linear-linear graph of Fig. 34A, equal distances along the axes refer to equal absolute changes. Is there any logical reason for preferring relative changes to absolute changes? The usual answer is that whereas the value of an absolute change Δx depends on the dimensions used, a relative change $\Delta x/x$ is dimensionless. True enough, but why not use a standard state to get rid of dimensions, instead of the prevailing state, which varies? Let us attempt to find a more satisfying answer by looking at a specific example: how a change in the enzyme concentration of reaction 2 affects the steady-state behaviour of the system described in Fig. 34.

Fig. 35A depicts two steady states for two different prevailing concentrations of enzyme 2: one at $e_2 = 2$ and one at $e_2 = 10$. The dotted curves around these two v_2-characteristics were calculated after increasing and decreasing the two e_2-values by 1 (i.e., equal absolute changes). The boxes show the magnitudes of the concomitant changes in J and $\bar{x}$. The problem with using absolute changes is apparent in Fig. 35A. The obvious difference in the size of the boxes is possibly a reflection of the relative insensitivity of the steady state at $e_2 = 10$ to changes in e_2, but in part it must also be a reflection of the fact that increasing $e_2 = 10$ to 11 represents a 1.1-fold change, while increasing $e_2 = 2$ to 3 represents a 1.5-fold change. These two changes, although equal in absolute value, cannot be regarded as having the 'same' effect on the enzyme concentration. Only when the same fold-change in $e_2 = 2$ is considered at both steady states (Fig. 35B), can one be sure that the effects on the steady state are not partly due to a difference in scale. Fig. 35B shows that changes around $e_2 = 10$

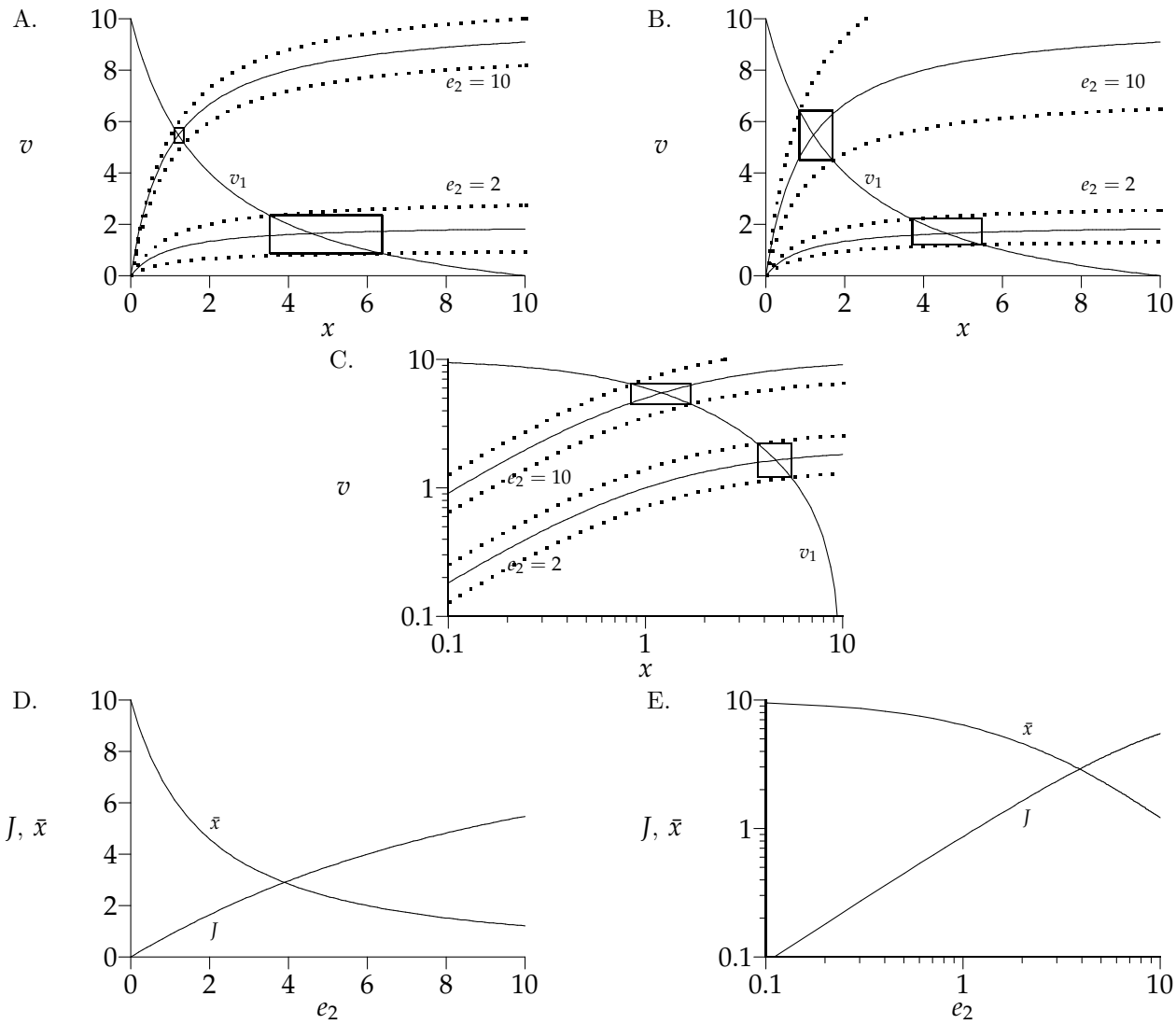

Figure 35: *Rate characteristics of the reactions in Fig. 33 with respect to the linking metabolite X* (A, B, and C) *and parameter portraits of steady-state variables with respect to variation in* e_2 (D and E). A, B. Unscaled rate characteristics. C. Log-log form of the rate characteristics. The rate equations for v_1 and v_2 were the same as those in Fig. 34; v_2 was calculated at a low enzyme concentration ($e_2 = 2$) and a high enzyme concentration ($e_2 = 10$) (solid lines). In (A) the dotted lines were calculated for a decrease and increase of 1.0 in the two e_2-values. In (B) and (C) the dotted lines were calculated for a 1.4-fold decrease and increase of the two e_2-values. D. A portrait of the steady-state response in J and $\bar{x}$ when parameter e_2 is varied continuously. E. Log-log form of the parameter portrait in (D).

have a greater effect on the flux and a smaller effect on $\bar{x}$ than changes around $e_2 = 2$. However, the above argument that the context in which any change is observed must be taken into account holds equally for these changes in J and $\bar{x}$. This point can be illustrated in relation to the need for considering pH-changes instead of changes in the hydrogen-ion concentration: for example, in the mammalian stomach at a hydrogen-ion concentration of 10^{-3} mol l^{-1} an increase of 10^{-5} mol l^{-1} would be virtually imperceptible and would be expected to have only slight consequences; on the other hand the same increase in a cell at a hydrogen-ion concentration of 10^{-7} mol l^{-1} might well be devastating. Here the comparison is between 1.01-fold and 100-fold changes, whereas if equal

pH-changes had been considered the fold-changes would have been equal. The crucial point is that in most circumstances we want to compare *changes* in state, rather than the states themselves, and a particular change can have very different effects in different contexts. Scaling the changes to a standard state does not get rid of this problem; standard states do not eliminate units, they just sweep them under the carpet.

Linear scales thus suffer fatally this scaling effect, whereas in logarithmic scales the scaling effect is absent. This then is the crucial difference between linear and logarithmic scales: if one compares a fixed distance at different points of a linear scale, one compares the same absolute change at the different points, whereas if one compares a fixed distance at different points of a logarithmic scale, one compares the same relative (or fractional or percentage) change at the different points. Cause-and-effect relationships are nearly always quantified in terms of changes, and it follows that for such changes to be compared they should be measured on a logarithmic scale. Up to now we have discussed the comparison of two values, but the argument also holds for continuous curves, which can be thought of as a set of closely lying data points: more often than not, such curves should be represented in log-log space in order for there slopes to be interpretable as sensitivities of responses.

The more appropriate representation of the rate characteristics is therefore in double-logarithmic space (Fig. 35C); the dotted curves were calculated for a 1.4-fold decrease and increase in each of the two concentrations of E_2 (i.e., equal relative changes). From this graph it is clear that the flux is more sensitive to changes in e_2 at 2 than at 10; just the opposite holds for $\bar{x}$, which is less sensitive at the lower e_2 (note that consideration of Fig. 35B led to the opposite conclusion).

A complete picture of the steady-state response to changes in e_2 is given in a *parameter portrait* (Fig. 35D). Imagine we change e_2 from 0.1 to 10 in a series of very small steps. For each step (i.e., for each value of e_2) we draw a rate characteristic as in Fig. 35A and determine the steady-state flux J and concentration $\bar{x}$. If we now plot the variation of J and $\bar{x}$ as a function of e_2 (the enzyme concentration we varied), the resulting graph (Fig. 35D) is called a parameter portrait, as it shows how changes in the parameter e_2 affect the steady-state behaviour of the system.

For the same reasons as above, the log-log representation of this graph (Fig. 35E) is the one to consider. The fact that e_2 is now varied continuously does not change the argument; we are still comparing different steady states, albeit a series that lie next to each other. Consider the flux J for values of e_2 up to about 2: doubling of e_2 leads to a doubling of the flux as the line as a slope of 1 (enzyme 2 may be said to be 'rate-limiting'); at higher e_2, however, line bends off and a doubling of e_2 will no longer cause a doubling in J.

With regard to $\bar{x}$, the difference between the unscaled and the scaled curves is more striking than with regard to the flux. If one assumes the definition of homeostasis as: '**Homeostasis** means that the change in level of a stationary metabolite is very low after parameter perturbation', one would deduce from Fig. 35D that $\bar{x}$ is not homeostatically maintained, except perhaps at high values of e_2. However, the above argument compels us to consider relative rather than absolute changes. From Fig. 35E it is clear that the relative change in $\bar{x}$ is small at low e_2, while at higher e_2 the relative change in $\bar{x}$ becomes inversely proportional to the relative change in e_2. If a definition of homeostasis in terms of small relative change is accepted, then it is clear that $\bar{x}$ is homeostatically maintained at *low* e_2 but not at *high* e_2.

9.3 Control and elasticity, and their relationships

In the previous lectures you were introduced to the rate characteristics that show the dependence of individual reaction rates v_1 and v_2 of the system in Fig. 33 on the concentration of X. We also concluded that the most informative form of these rate characteristics is their log-log form, because equal distances on a log-scale represent equal fractional changes (or, similarly, percentage changes). We concluded that, for the purpose of comparing the responses of different steady states to perturbations, we need to compare fractional changes, both in the parameter perturbations and in the steady-state responses.

We are now going to explore the quantitative behaviour of the system around the steady state. In the process we shall develop a definition of the concept of *control* and discover its relationship to certain intrinsic properties of the individual reactions that comprise the system, which we call the *elasticities* of the reactions.

Let us return to the log-log rate characteristic and zoom in on the steady state until the rate curves around it are for all practical purposes linear (Fig. 36A). We now consider an increase in the rate of reaction 1 by a fractional amount $d \ln v_1$. The only way to cause such an increase is by changing a parameter that affects reaction 1 only. If reaction 1 is enzyme-catalysed a convenient way of doing this is by increasing the enzyme concentration. The rate increase is shown in Fig. 36B as an upward shift in the v_1-curve by a distance $\overline{\text{ab}}$. This perturbation has two important characteristics: it is *independent* and *local* (because the direct effect of the parameter change is on one reaction rate only).

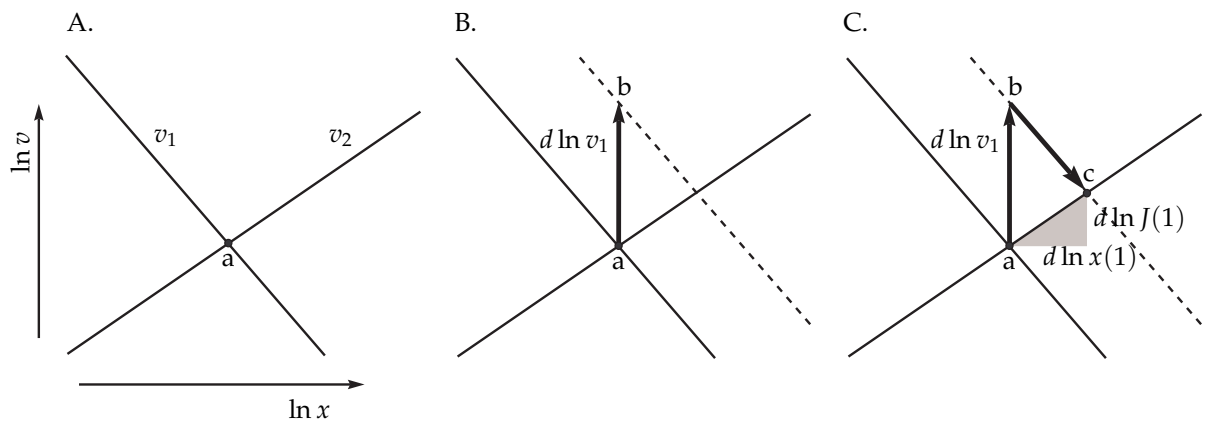

Figure 36: *Quantifying the effect on the steady state of a perturbation of the rate v_1 of the system in Fig. 33.* A. Highly magnified view of the rate characteristics around the steady state at point a. At such magnification the rate curves are linearised. B. The rate v_1 is initially perturbed by the fractional amount $d \ln v_1$ (equal to the length $\overline{\text{ab}}$) by modulating any parameter that affects only reaction 1, such as the concentration of enzyme 1. A rate perturbation with these characteristics is defined as *independent* and *local*. C. The system then relaxes to the new steady state at point c where the two rates are again equal. The local rate change $d \ln v_1$ therefore results in a steady-state flux change $d \ln J(1)$ and concentration change $d \ln x(1)$.

After the perturbation the system is of course thrown out of steady state. Reaction 1 is now faster than reaction 2 so that there is net production of the intermediate X, which in turn increases the rate

of reaction 2 until the two rates match each other in a new steady state. This is shown in Fig. 37C by the arrow $\overline{\text{bc}}$. Make sure that you understand the difference between Figs. 37B and 37C: B describes a *local* rate perturbation, while C describes a *systemic* relaxation to a new steady state that follows the initial rate perturbation (systemic because the position of the new steady state depends on both rate characteristics, and not only on one). Another way of looking at it is that while the effect in B depends only on the properties of reaction 1, the new steady state depends on the properties of both reactions. The shift from the old steady state to the new is accompanied by a flux change $d\ln J(1)$ and a concentration change $d\ln x(1)$ (the bracketed 1 indicates that these changes in the steady-state variables are caused by a change in the local rate of reaction 1). We can therefore say that the local rate change $d\ln v_1$ *caused* the systemic steady-state changes $d\ln J(1)$ and $d\ln x(1)$.

9.3.1 Control coefficients

We are now in a position to define a quantitative measure of control as the ratio of a systemic change (such as $d\ln J(1)$ or $d\ln x(1)$) and the local rate change that caused it ($d\ln v_1$). We call this ratio a **control coefficient**. Depending on which steady-state variable we are considering we can define a *flux-control coefficient*

$$C^J_{v_1} = \frac{d\ln J(1)}{d\ln v_1} \tag{130}$$

or a *concentration-control coefficient*

$$C^x_{v_1} = \frac{d\ln x(1)}{d\ln v_1} \tag{131}$$

Often, the subscript of a control coefficient is simplified to just the number so that, for example, $C^J_{v_1}$ becomes C^J_1.

If we remember that a log-change $d\ln x$ can also be written as a fractional change dx/x, then we can recast these definitions as

$$C^J_{v_1} = \frac{dJ/J}{dv_1/v_1} \tag{132}$$

and

$$C^x_{v_1} = \frac{dx/x}{dv_1/v_1} \tag{133}$$

If both numerator and denominator are multiplied by 100 the control coefficients can be seen to be *ratios of percentage changes*. In fact, this definition leads to a simple *operational definition of control coefficients* as the % change in J or x when the local rate of reaction 1 is perturbed by 1%.

Up to now we considered a change in the local rate of reaction 1 and its effects on the steady state. In exactly the same way we can consider an increase in the local rate of reaction 2. This is shown in Fig. 37B (compare with Fig. 37A which again shows the effects of a change in v_1). The new steady state is at point d on the graph.

Just as before we can quantify the response of the steady state to the perturbation in v_2 in terms of the control coefficients

$$C^J_{v_2} = \frac{d\ln J(2)}{d\ln v_2} \tag{134}$$

and

$$C^x_{v_2} = \frac{d\ln x(2)}{d\ln v_2} \tag{135}$$

What would happen if we made *equal* fractional changes in the rates of both reaction 1 and reaction 2? This is depicted in Fig. 37C; the new steady state is at point b. Two relationships are

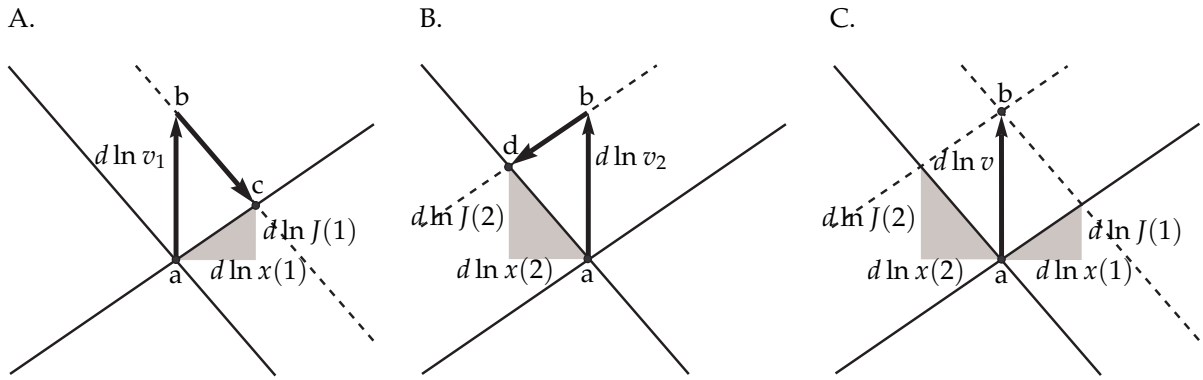

Figure 37: *A geometrical interpretation of a control coefficient.* In (A) the rate v_1 is initially perturbed by the amount $d\ln v_1$, after which the system settles into the new steady state at point c. In (B) the rate v_2 is perturbed by the same amount $d\ln v_2 = d\ln v_1$, after which the system settles into the new steady state at point d. As discussed in the text the ratios $d\ln J(1)/d\ln v_1$ and $d\ln J(2)/d\ln v_2$ are respectively equal to the flux-control coefficients C_1^J and C_2^J, while the ratios $d\ln x(1)/d\ln v_1$ and $d\ln x(2)/d\ln v_2$ are respectively equal to the concentration-control coefficients C_1^x and C_2^x. In (C) both rates are perturbed by the same fractional amount $d\ln v$ so that the eventual steady state is at point b, which can clearly be seen to be the sum of the individual perturbations made in (A) and (B).

intuitively clear (you can prove it using simple high-school geometry): (i) The fractional change in flux (denoted by the arrow $\overline{\text{ab}}$) is the sum of the individual flux-changes $d\ln J(1)$ and $d\ln J(2)$; (ii) there is no change in the steady-state concentration of X because the fractional changes $d\ln x(1)$ and $d\ln x(2)$ are equal and opposite in sign and therefore cancel.

This leads to two important properties of control coefficients, which are quite general: for any flux, the control coefficients of all the steps in a system sum to one (the **flux-control summation property**); for any variable concentration, the control coefficients of all the steps in a system sum to zero (the **concentration-control summation property**). For this system these equations are:

$$C_1^J + C_2^J = 1 \tag{136}$$

and

$$C_1^x + C_2^x = 0 \tag{137}$$

It is easy to see from Fig. 37C where these equations come from:

$$d\ln v = d\ln J(1) + d\ln J(2) \tag{138}$$

Therefore, because $d\ln v = d\ln v_1 = d\ln v_2$,

$$\frac{d\ln v}{d\ln v} = \frac{d\ln J(1)}{d\ln v_1} + \frac{d\ln J(2)}{d\ln v_2} \tag{139}$$

or, using the definitions of the flux-control coefficients,

$$C_1^J + C_2^J = 1$$

Similarly, because $d \ln x(1) = -d \ln x(2)$,

$$d \ln x(1) + d \ln x(2) = 0 \tag{140}$$

Dividing by $d \ln v_1 = d \ln v_2$ gives

$$\frac{d \ln x(1)}{d \ln v_1} + \frac{d \ln x(2)}{d \ln v_2} = 0 \tag{141}$$

or, using the definitions of the concentration-control coefficients,

$$C_1^x + C_2^x = 0$$

The summation properties have important implications for biological systems:

1. Although the flux-control summation property (Eq. 136) states that the flux-control coefficients of all steps in a metabolic pathway must add up to one, there is no necessity for the classical, but erroneous notion that a pathway *must* contain a 'rate-limiting step' (which would have total control of the flux—a flux-control coefficient of one—while all other steps would have to have zero flux-control coefficients in that case). Such a fixed control profile is virtually never a feature of realistic systems; in general, control is distributed through the system and the control profile varies with the steady state. In other words:

   Reactions share the control of a flux.

   What is clear is that if the rates of all steps in a system are increased by the same factor, then the flux must also increase by the that factor.

2. The concentration-control summation property (Eq. 137) states that for any variable concentration the concentration-control coefficients of all steps in a metabolic pathway will add up to zero. This means that an increase in the activity of some steps will increase the variable concentration, whereas an increase in the activity of other steps will decease the variable concentration. If the activity of all steps in the pathway increases by the same percentage (i.e., the same fractional change), the variable concentration will stay the same. The concentration-control summation property can be summarised as

   Whereas some reactions act to decrease metabolite concentrations, others increase them.

9.3.2 Relating systemic effects to local effects: the elasticity coefficient

Now that we have a rigorous and quantitative way of defining control, we are in a position to ask some crucial questions: Is it possible to relate the values of the control coefficients to the local properties of the reactions? If so, which local properties are the ones we should consider? Philosophically, the answers to these questions are very important: if this can be done it means we can relate properties of the system as a whole to the properties of the parts of the system. This must ultimately form the basis of any understanding of the workings of living systems and is therefore a deep matter that any biochemist must consider.

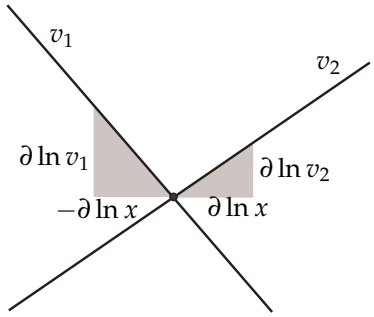

Figure 38: *The elasticities of the reactions at the steady-state point.* The slope of each rate characteristic is seen to be the ratio $\partial \ln v / \partial \ln x$. These slopes are called *elasticity coefficients* and are identical to the *kinetic orders* of the reactions. Partial derivatives (denoted by the symbol ∂) are used to denote that the effect of only a change in x on the rates is considered; all the other reaction parameters are kept constant.

Let us return to the graph in Fig. 37C. It should be clear that the steady-state responses to perturbations in either local rate are completely determined by the slopes of the rate characteristics around the steady state. Let us redraw the graph in Fig. 37C so as to focus on the individual rates, rewriting the quantities as local reaction quantities (see Fig. 38).

For reaction 1 the slope of its rate curve at the steady-state point is a ratio that we call the **elasticity coefficient** of reaction 1 with respect to the concentration of X:

$$\varepsilon_x^{v_1} = \frac{\partial \ln v_1}{-\partial \ln x} \tag{142}$$

Similarly, the *elasticity coefficient* of reaction 2 with respect to the concentration of X is defined as

$$\varepsilon_x^{v_2} = \frac{\partial \ln v_2}{\partial \ln x} \tag{143}$$

We need to use partial changes and partial derivatives to denote that we only change x while keeping all the other reaction parameters (such as kinetic constants, other substrates, products and effectors) constant.

Again we can recast these definitions in terms of fractional or percentage changes:

$$\varepsilon_x^{v_1} = \frac{\partial v_1 / v_1}{-\partial x / x} \tag{144}$$

and

$$\varepsilon_x^{v_2} = \frac{\partial v_2 / v_2}{\partial x / x} \tag{145}$$

We can also give an operational definition of an elasticity as the %-change in the reaction rate that follows a 1%-change in one of the reaction parameters.

Let us now see whether we can use the information in Figs. 37 and 38 to find a mathematical relationship between control and elasticity coefficients. We start with the definition of the flux-control coefficient of reaction 1:

$$C_{v_1}^J = \frac{d \ln J(1)}{d \ln v_1} \tag{146}$$

We know from Eq. 138 that $d\ln v_1 = d\ln J(1) + d\ln J(2)$ so that

$$C^J_{v_1} = \frac{d\ln J(1)}{d\ln J(1) + d\ln J(2)} \tag{147}$$

Dividing both numerator and denominator with $d\ln x = d\ln x(1) = -d\ln x(2)$ gives

$$C^J_{v_1} = \frac{\frac{d\ln J(1)}{d\ln x}}{\frac{d\ln J(1)}{d\ln x} + \frac{d\ln J(2)}{d\ln x}} \tag{148}$$

Comparing Figs. 37 and 38 we see that $d\ln J(1) = \partial\ln v_2$ and $d\ln J(2) = \partial\ln v_1$. Also, $d\ln x = \partial\ln x$. Inserting these equivalencies gives:

$$C^J_{v_1} = \frac{\frac{\partial\ln v_2}{\partial\ln x}}{\frac{\partial\ln v_2}{\partial\ln x} + \frac{\partial\ln v_1}{\partial\ln x}} \tag{149}$$

In order to get to elasticities we only need to make one further change:

$$C^J_{v_1} = \frac{\frac{\partial\ln v_2}{\partial\ln x}}{\frac{\partial\ln v_2}{\partial\ln x} - \left(\frac{\partial\ln v_1}{-\partial\ln x}\right)} \tag{150}$$

Now, using the definitions of elasticities given in Eqs. 142 and 143 above, we obtain the relationship we seek:

$$C^J_{v_1} = \frac{\varepsilon^{v_2}_x}{\varepsilon^{v_2}_x - \varepsilon^{v_1}_x} \tag{151}$$

It is left to you to derive the other relationships between control and elasticity coefficients:

$$C^J_{v_2} = \frac{-\varepsilon^{v_1}_x}{\varepsilon^{v_2}_x - \varepsilon^{v_1}_x} \tag{152}$$

$$C^x_{v_1} = \frac{1}{\varepsilon^{v_2}_x - \varepsilon^{v_1}_x} \tag{153}$$

$$C^x_{v_2} = \frac{-1}{\varepsilon^{v_2}_x - \varepsilon^{v_1}_x} \tag{154}$$

Above we discovered the important summation properties of control coefficients (Eqs. 136 and 137). Using the information in Figs. 37 and 38 we can also derive another set of important properties called **connectivity properties**. From the graphs it should be clear that:

$$\frac{d\ln J(1)}{d\ln J(2)} = \frac{\partial\ln v_2}{\partial\ln v_1} \tag{155}$$

Using the same equivalencies that were used above, dividing both numerator and denominator on the left hand side by $d\ln v$, and dividing both numerator and denominator on the right hand side by $\partial\ln x$, this equation can be transformed to

$$\frac{\frac{d\ln J(1)}{d\ln v_1}}{\frac{d\ln J(2)}{d\ln v_2}} = \frac{\frac{\partial\ln v_2}{\partial\ln x}}{-\left(\frac{\partial\ln v_1}{-\partial\ln x}\right)} \tag{156}$$

Using the definitions of control and elasticity coefficients we obtain

$$\frac{C^J_{v_1}}{C^J_{v_2}} = -\frac{\varepsilon^{v_2}_x}{\varepsilon^{v_1}_x} \tag{157}$$

which can be rewritten as

$$C^J_{v_1}\varepsilon^{v_1}_x + C^J_{v_2}\varepsilon^{v_2}_x = 0 \tag{158}$$

which is called the **flux-connectivity property**.

There is also a **concentration-connectivity property**

$$C^x_{v_1}\varepsilon^{v_1}_x + C^x_{v_2}\varepsilon^{v_2}_x = -1 \tag{159}$$

It can derived using a similar procedure as above, but in the context of a slightly different thought experiment. This derivation will not be given here.

9.4 The steady-state response to a parameter perturbation: the response coefficient

In our thought experiments which led to the definition of a control coefficient one aspect was glossed over: we had to change a parameter in order to cause a local rate change, but the relationship between this parameter change and the ultimate response in the steady state was left unexplored. We have, however, already encountered this relationship a previous lecture (Fig. 35) where we considered the steady-state response to changes in the enzyme concentration e_2. We want a quantitative formulation of this response, and, as before, we consider a ratio of fractional changes. If we consider the response in the steady-state flux to, for example, a change in the enzyme concentration e_2 we define the **flux-response coefficient** as

$$R^J_{e_2} = \frac{d\ln J}{d\ln e_2} \tag{160}$$

Similarly, if we consider the response in the steady-state concentration x to a change in the enzyme concentration e_2 we obtain a **concentration-response coefficient**

$$R^x_{e_2} = \frac{d\ln x}{d\ln e_2} \tag{161}$$

More generally, for any steady-state variable y and any parameter p

$$R^y_p = \frac{d\ln y}{d\ln p} \tag{162}$$

which can be recast in terms of fractional changes

$$R^y_p = \frac{dy/y}{dp/p} \tag{163}$$

Again these coefficients can be seen to be *ratios of percentage changes*, so that the response coefficient can be defined operationally as the % change in y when the parameter p is perturbed by 1%.

We can define response coefficients with respect to any system parameter. It should be clear that response coefficients are the quantities that we measure experimentally.

If you return to the *log-log parameter portraits* in Fig. 35E it should be clear that for any specific value of e_2 the slope of the $\ln J$-curve equals the response coefficient $R^J_{e_2}$, while the slope of the $\ln x$-curve equals the response coefficient $R^x_{e_2}$.

9.5 The partitioned response property

In the previous section we asked how the steady state responds to a change in a system parameter, i.e., to what degree each parameter controls the steady state. The effects of a parameter change must always originate at a specific reaction step (assuming the parameter directly affects only one step). It should therefore come as no surprise that the steady-state response to a parameter perturbation is always a combination of two effects: the direct and immediate effect of the parameter change on the local rate of the reaction to which it is specific, and the subsequent effect of this local rate change on the steady state. This is called the **partitioned response property** (also called the *combined response property*). So, if we make a perturbation in parameter that affects only v_1—say, the enzyme concentration e_1—the effect of its perturbation on a steady-state variable such as J must be propagated through a local rate change in v_1.

$$\frac{d\ln J}{d\ln e_1} = \frac{d\ln J}{d\ln v_1} \cdot \frac{\partial \ln v_1}{\partial \ln e_1} \tag{164}$$

or, in terms of coefficient symbols,

$$R^J_{e_1} = C^J_{v_1}\varepsilon^{v_1}_{e_1} \tag{165}$$

The partitioned response equation relates the response coefficient $R^J_{e_1}$ to the control coefficient $C^J_{v_1}$ and the elasticity coefficient $\varepsilon^{v_1}_{e_1}$.

Partitioned response equations can be written for any parameter p and any steady-state variable y. If p acts only through reaction i then

$$R^y_p = C^y_{v_i}\varepsilon^{v_i}_p \tag{166}$$

Note that, according to our definition, a control coefficient is a property of a step in the system, and should be parameter-independent. This follows from a rearrangement of Eq. 166:

$$C^y_{v_i} = \frac{R^y_p}{\varepsilon^{v_i}_p} = \frac{d\ln y/d\ln p}{\partial \ln v_i/\partial \ln p} \tag{167}$$

where p, the parameter which affects v_i, cancels from the equation.

From our definitions of control and response coefficients it should be clear that they are conceptually distinct from each other, a control coefficient being associated with a step, while a response coefficient is associated with a parameter. In especially the earlier control analysis literature this distinction was seldom made, the term control coefficient being used for both control and response coefficients. The main reason for this was that they are often identical. In the present example, the elasticity coefficient $\varepsilon^{v_1}_{e_1}$ is equal to 1 (*why??*) so that $R^y_{e_1} = C^y_{v_1}$.

We now have all the tools in our toolbox to arrive at a *functional description of metabolic regulation*. The next section starts by introducing the classical textbook view of metabolic regulation, and then shows how this view fails to provide a functional description.

10 Classical view of metabolic regulation *vs.* functional view of metabolism

10.1 Metabolic regulation: the textbook view

When you look at any modern textbook of biochemistry, the regulation of metabolic pathways is usually discussed in terms of allosteric feedback or feedforward loops, which make certain "pacemaker" enzymes sensitive to the concentration of metabolic end-products or intermediates. These pacemaker enzymes are classified as "rate-limiting steps" and are said to regulate the flux through the pathway.

A typical biosynthetic pathway is shown in Fig 39. Such a pattern is generally observed in the biosynthesis of, for example, amino acids or nucleotides. A feature of such pathways is that the first committing enzyme in a branch is usually far from equilibrium (and often designated "irreversible"), and inhibited allosterically by the end-product of its branch (as indicated by the dotted arrows). The other enzymes in a branch are close to equilibrium.

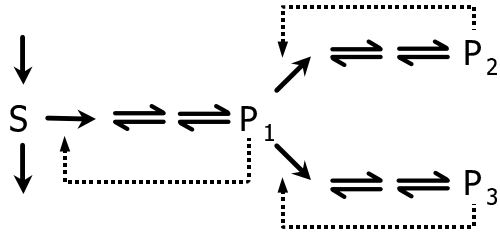

Figure 39: *A typical biosynthetic pathway of products* P_2 *and* P_3 *from substrate* S. P_1 is an intermediate product at a branch point and acts as a substrate for the biosynthetic branches for P_2 and P_3. Each product inhibits the first committing enzyme in its branch by allosteric end-product (feedback) inhibition.

The classical view of metabolic regulation in a pathway such as depicted in Fig. 39 can be summarised as follows:

- the non-equilibrium steps (denoted by single "one-way" arrows) are rate-limiting;
- allosteric end-product inhibition enables the non-equilibrium enzymes to regulate the flux through the pathway; and
- the near-equilibrium steps (denoted by double "two-way" arrows) transmit the flux effectively.

Look up for yourself how the regulation of glycolysis is described in a biochemistry textbook (e.g. *Voet & Voet, Biochemistry, 2nd edition*: p. 471–474). Note in particular the following sentences:

> The metabolic flux through an entire pathway is determined by its rate-determining step (or steps) which, by definition, is much slower than the following reaction step(s). (p. 471)
>
> *PFK, an elaborately regulated enzyme functioning far from equilibrium, evidently is the major control point for glycolysis in muscle under most conditions.* (p. 472)

To illustrate that this description is not limited to one textbook, consider these quotes from other biochemistry texts:

> In every metabolic pathway there is at least one reaction that, in the cell, is far from equilibrium because of the relatively low activity of the enzyme that catalyzes it (Fig. 14-16). The rate of this reaction is not limited by substrate availability, but only by the activity of this enzyme. The reaction is therefore said to be *enzyme-limited,* and because its rate limits the rate of the whole reaction sequence, the step is called the *rate-limiting step* in the pathway. In general, these rate-limiting steps are very exergonic reactions and are therefore essentially irreversible under cellular conditions. (p. 427)
>
> Lehninger, A.L., Nelson, D.L. and Cox, M.M. (1993)
> *Principles of Biochemistry* (2nd edn.) Worth, New York,

> The phosphorylation of fructose 6-phosphate is highly exergonic and irreversible, and **phosphofructokinase**, the enzyme that catalyzes it, is the key enzyme in glycolysis. (p. 348)
>
> Campbell, M.K. (1995)
> *Biochemistry* (2nd edn.) Saunders College Publishing, Philadelphia

What is the problem with these descriptions? They are blatantly wrong! (No, we do not make this statement out of idle arrogance—there is hard experimental evidence to corroborate our statement). Although many textbooks claim that phosphofructokinase controls glycolytic flux, a crucial experiment was done by Heinisch in 1986 in the laboratory of Walter Zimmerman. He over-expressed phosphofructokinase to 3.5× its normal level in yeast cells, and yet there was absolutely **no increase** in the flux from glucose to ethanol! If phosphofructokinase had been rate-limiting, an increase in the amount of this enzyme should surely have led to a higher glycolytic flux. This study was followed up by Irene Schaaf in the same laboratory who over-expressed, singly or in pairs, eight different yeast glycolytic enzymes, again with no significant effect on either the rate of ethanol production or the concentrations of "key" metabolites. The glycolytic enzymes therefore seem to have no control over the flux through their own pathway.

Clearly, current textbook wisdom on metabolic regulation is flawed. The aim of this course is to provide you with an alternate view of metabolic control and regulation, a view that is consistent with experimental results. Such an integrated view of cellular function will require you to think critically and broadly, and it is at the cutting edge of research in this field. The importance and impact of this view was recently emphasised by a commentator (Prof Steve Oliver of Manchester University, UK) in the influential science journal *Nature*, who said:"It could mean that biologists in the 21st century need a re-think of their view of cellular economy that is every bit as radical as that initiated for political economy by John Stuart Mill and William Stanley Jevons in the 19th century".

The development of such a new integrative description of cellular regulation is a main research focus of the Laboratory for Molecular Systems Biology (Profs Jannie Hofmeyr, Johann Rohwer, Jacky Snoep, and Dr Dawie van Niekerk) in our department. In this course we shall therefore not only teach you new concepts, but also give you a flavour of what biochemical research in this field is like.

10.2 The functional view of metabolism

Although the regulation of metabolic pathways is usually discussed as if the pathway existed in isolation (such as in Fig. 39), there is a general consensus among biochemists about how metabolism as

a whole is functionally linked. You were introduced to an overview of metabolism in the Biochemistry 244 course—go back to the module on Integration of Metabolism. Such a functional view is summarised in Fig 40.

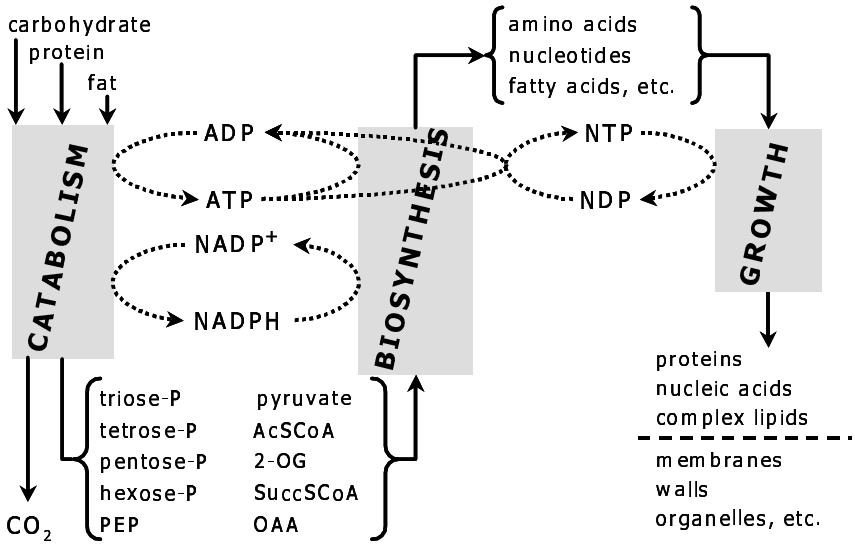

Figure 40: *Functional organisation of metabolism.* The primary energy sources are degraded in catabolic pathways to form a series of metabolic intermediates (sugar phosphates, activated CoA-intermediates, PEP, pyruvate, oxaloacetate and 2-oxoglutarate) and carbon dioxide. Catabolism also produces ATP and reducing equivalents (NADPH). The 3C–6C intermediates, NADPH and ATP are then used in biosynthetic reactions to produce amino and fatty acids, and nucleotides. Finally, during growth, these building blocks are used to form macromolecules (proteins, nucleic acids, lipids) and higher-order cellular structures, a process that also requires an input of free energy (NTP).

Although this functional organisation of metabolism and the classical view of metabolic regulation are both found in textbooks, the classical view of regulation largely ignores the functional view. In the functional organisation (Fig. 40) the different catabolic and anabolic metabolic blocks are linked by intermediates. Thus, biosynthesis communicates with catabolism through ATP, ADP, $NADP^+$, NADPH, and the 3C–6C small molecules, and growth communicates with biosynthesis through the amino acids, fatty acids and nucleotides, as well as NTP and NDP. Understanding this "communication" is crucial for understanding metabolic function, and yet this aspect is sorely missing from the classical view in Fig. 39: it simply considers the biosynthetic pathway in isolation without asking what happens to the products. We have likened it to an economic analysis of a factory that ignores the consumption of the products of the factory, i.e. trying to understand the economics in terms of the supply only, without taking demand into consideration.

The crucial concept thus is that metabolic blocks communicate with each other through intermediates. To understand how this works, we shall further on in the course consider the simplest case: two coupled reactions (or reaction blocks) with one common intermediate.

11 Supply-demand analysis of metabolic systems

We now have all the necessary tools in our arsenal to re-examine our concepts of metabolic regulation: rate characteristics, thermodynamics (ΔG, ΔG°, Γ and K_{eq}), enzyme kinetics (reversible Michaelis-Menten, competitive and uncompetitive inhibition, reversible Hill kinetics), and metabolic control analysis. In this section we put everything together to arrive at a functional view of metabolic regulation in supply-demand systems.

11.1 The factory analogy

Let us start by presenting the following analogy: how economical would a factory be if it ignored the consumer demand for its product, continuing, for example, to produce product in spite of a low demand for the product? It is easy to see that for the factory to survive, its production should match the demand as it varies from high to low. Put differently, the demand should control the production rate in the factory. If this were not the case, the factory could be caught unawares on two accounts: (i) either it cannot supply sufficient product to meet the demand, in which case competitors will take over and drive it out of business, or (ii) it will over-produce, leading to accumulation of half-finished products on the assembly line, which is clearly also uneconomical.

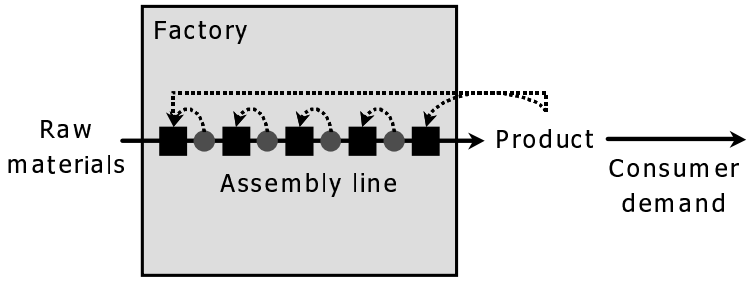

Figure 41: *The factory analogy for supply and demand.* A factory cannot function efficiently unless its output is controlled by the demand for product. There has to be a feedback down the production line (indicated by the dotted lines). The dark grey circles indicate the product in various intermediate states of completion; the black squares indicate different machines in the production process.

If the consumer demand is to control the factory's production rate, there needs to be feedback from the product levels to the assembly line (Fig. 41). This can work either by each intermediate product in the production process shutting down the machine immediately in front of it if it accumulates, or by the final product shutting down the very first machine in the assembly line if it accumulates, thus shutting down the whole factory (see the dotted lines in the above figure).

In the following sections we will transfer the factory analysis to a biosynthetic metabolic pathway. We will see how the demand for end-product needs to control the flux through the pathway if it is to function effectively. We will also discover how various means of regulation differ in their efficacy.

11.2 Regulating the cellular economy of supply and demand

Refer to the PDF copy of the scientific paper:
J.-H.S. Hofmeyr and A. Cornish-Bowden (2000) Regulating the cellular economy of supply and demand *FEBS Lett.* **476,** 47–51.

11.3 Moulding the supply rate characteristic: from thermodynamic to kinetic regulation

Consider the following linear supply-demand system around the metabolic intermediate P. Note that the supply consists of three steps converting S to P.

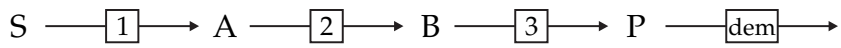

Figure 42: *A linear metabolic supply-demand system.* The biosynthetic supply consists of three steps, which convert the substrate S to the product P via the intermediates A and B. The demand is a single reaction consuming P.

We have seen that a sufficient condition for the demand to control the flux through such a system (C^J_{demand}=1) is that $\varepsilon_p^{v_{demand}} = 0$ (see Eq. 8 and Fig. 4 in the FEBS Letters paper). Put into words, this means that the demand reaction should be saturated with P, i.e. the concentration of P should be far above the K_m of the demand reaction for P. Assume that the demand reaction is catalysed by irreversible Michaelis-Menten kinetics, i.e.

$$v_{demand} = \frac{V_{demand} \cdot p}{K_p + p} \tag{168}$$

where V_{demand} is the maximal reaction rate of the demand. If $p \gg K_p$ then $v_{demand} \approx V_{demand}$ (the rate equals the maximal velocity of the enzyme) and the rate will be insensitive to changes in p. Hence, at these concentrations of P, the graph of $\ln v_{demand}$ vs. $\ln p$ will approach a horizontal line with slope zero and $\varepsilon_p^{v_{demand}} = 0$.

We have also seen that if the demand controls the flux, then the supply determines the steady-state concentration of P (Figs. 4B and 4C in the FEBS paper). The steeper the supply rate characteristic (i.e., the more negative $\varepsilon_p^{v_{supply}}$) the less $\bar{p}$ will change if the demand is varied. If p is maintained in a narrow band, we say that the concentration is *homeostatically maintained,* or there is *homeostasis* in p. Homeostatic maintenance of end-products is desirable for a living cell, as these products are required in demand reactions and should always be available (e.g., amino acids should be available for protein synthesis or nucleotides for nucleic acid synthesis). In terms of the factory analogy, there should always be product available to fulfil the consumer demand.

So, if *flux control* is a function of the *demand block,* then *homeostasis* in $\bar{p}$ is a function of the *supply block.* We will now investigate how different kinetics for the supply enzymes affect their capacity to maintain $\bar{p}$ homeostatically.

11.3.1 Effect of supply block kinetics

Fig. 43 shows four different supply rate characteristics for the supply block (enzymes 1, 2 and 3) of the system in Fig. 42. The line labelled "mass action" shows the rate characteristic when all three enzymes follow mass-action kinetics. The K_{eq} values for the three enzymes are 400, 10 and 10 respectively. From Eq. 52 we know that the overall K_{eq} from S to P is 40000 ($400 \times 10 \times 10$). Hence, if $s = 1$, the supply block will be at equilibrium when $p = 40000$. This can be clearly seen on the graph, as v_{supply} approaches zero as p approaches 40000.

The line labelled "competitive" refers to the situation when all the supply enzymes follow reversible Michaelis-Menten kinetics (see Eq. 104). Recall that in the reversible Michaelis-Menten equation there is built-in competitive inhibition of the reaction rate by its product. Hence, enzyme 1 is

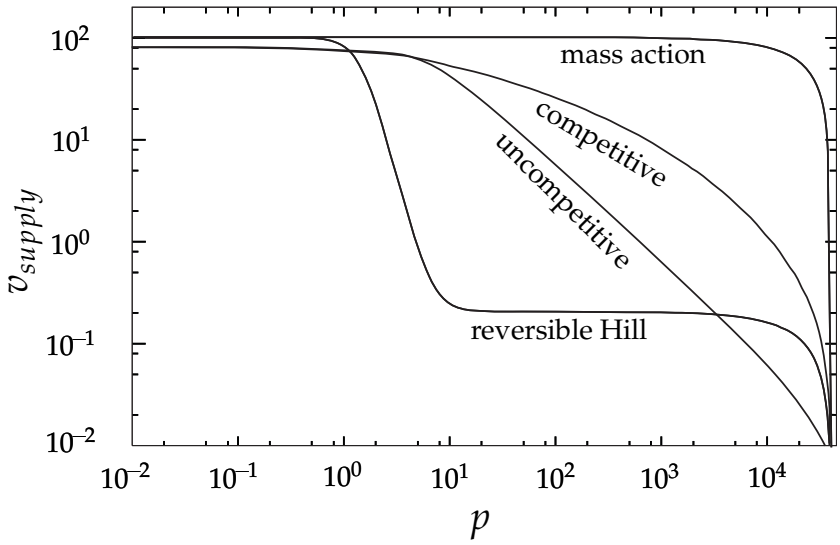

Figure 43: *The effect of different enzyme kinetics on the supply rate characteristic of the pathway in Fig. 42.* The lines on the graph are labelled with the kinetic types and are further discussed in the text.

competitively inhibited by A, enzyme 2 by B, and enzyme 3 by P. Fig. 43 clearly shows how the shape of the enzyme-catalysed rate characteristic differs from the mass-action case.

Uncompetitive inhibition changes the shape of the supply rate characteristic even more, in that its slope becomes steeper. The line labelled "uncompetitive" refers to the case where enzyme 3 is inhibited *uncompetitively* by its product P (see Eq. 114). Enzymes 1 and 2 follow ordinary reversible Michaelis-Menten kinetics as in the previous case.

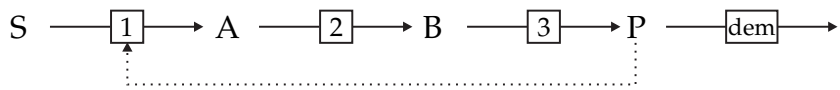

Figure 44: *The same supply-demand system as in Fig. 42, with a feedback loop.* P is an allosteric inhibitor of the first enzyme in the pathway.

The last line labelled "reversible Hill" refers to the case where enzyme 1 follows reversible Hill kinetics (see Eq. 119). Here, P is an allosteric modifier of enzyme 1. The "reversible Hill" graph thus illustrates the effect of introducing a feedback loop from P to the first enzyme, as indicated in Fig. 44.

Note that, for enzyme 1, P is now the modifier, S is the substrate and A is the product. In Eq. 119 (the generically defined equation), we called S the substrate, P the product and X the modifier. Do not let this confuse you! If you want to translate the generic Hill equation to the specific case in Fig. 44, you will have to keep in mind that A now plays the role of P, whereas P plays the role of X.

Two important observations are evident from the "reversible Hill" graph. (i) Over a certain range (for p between 1 and 10), the slope of the curve is steeper than all the others. This is *kinetic regulation* of the enzyme activity by P. (ii) However, the kinetic regulation has its limits: as p increases to values above 10, the rate characteristic jumps to the right and joins the mass-action curve. This is called *thermodynamic regulation*.

Finally, note that in all four the above cases the equilibrium constants for the three supply reac-

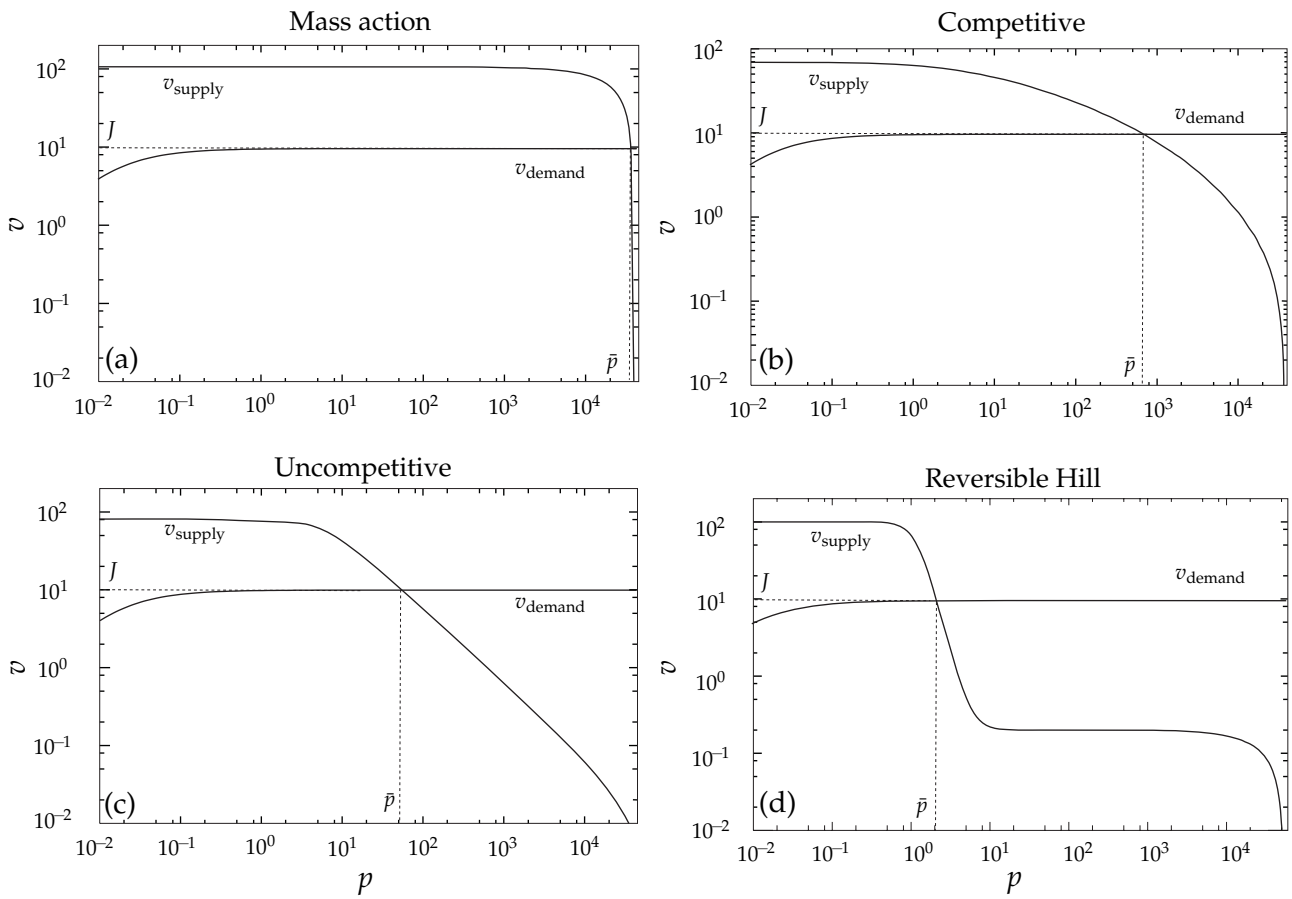

Figure 45: *The effect of supply enzyme kinetics on the steady state.* The rate characteristics are from Fig. 43, now drawn on separate graphs. Each graph includes the same demand rate characteristic. The steady-state flux (J) and concentration of P ($\bar{p}$) are indicated in each case.

tions are the same. You can see this as the supply tends towards equilibrium: all the supply rates approach zero as p approaches its equilibrium value of 40000.

11.3.2 Effect on the steady state

How will the different supply rate characteristics affect the steady state? Fig. 45 re-draws the supply rate characteristics on separate graphs, and includes the same demand rate characteristic on each one of them. The demand follows irreversible Michaelis-Menten kinetics with a K_p of 0.01 and a V_{demand} of 10. The steady-state flux and concentration of P are indicated with dotted lines.

Fig. 45(a) shows that for the mass-action case, although the demand controls the flux, this is so at very high (near-equilibrium) steady-state levels of $\bar{p}$. The cell would never be able to maintain such high concentrations of all its intermediates, as its solvent capacity is limited (there is simply not enough cellular water to dissolve all these metabolites!). Hence, this *thermodynamic regulation* of $\bar{p}$ by mass action is clearly insufficient for effective metabolic functioning.

How does the picture change when we assume that the reactions are enzyme-catalysed? Figs. 45(b) and (c) show the case of competitive and uncompetitive inhibition by P. In both cases, the demand still controls the flux, but now $\bar{p}$ is brought down by almost two orders of magnitude if we allow

for competitive inhibition. Uncompetitive inhibition decreases $\bar{p}$ by another order of magnitude and brings this concentration into a range that the cell can handle. Remember also, that the steepness of the supply rate characteristic determines homeostasis of $\bar{p}$ in the face of varying demand. The slope of the supply curve at the steady state in Fig. 45(c) is steeper than that in Fig. 45(b), indicating that uncompetitive inhibition allows for better homeostasis in $\bar{p}$. Although the supply slopes in Figs. 45(b) and (c) are both less than that in Fig. 45(a), $\bar{p}$ is now maintained at levels well below the equilibrium concentration, which is essential for cellular function as explained above. This effect is called *kinetic regulation* of $\bar{p}$.

What is the effect of introducing a feedback loop from P to enzyme 1? Fig. 45(d) shows that $\bar{p}$ is decreased even further. Moreover, the supply rate characteristic is steeper than in Fig. 45(c), indicating even better homeostasis in $\bar{p}$. Remember that the slope of the supply rate characteristic equals $\varepsilon_p^{v_{supply}}$. For uncompetitive inhibition, $\varepsilon_p^{v_{supply}}$ can be at most -1, whereas for allosteric feedback inhibition, $\varepsilon_p^{v_{supply}}$ can decrease to $-h$, where h is the Hill coefficient (in Fig. 45(d), $h = 4$).

11.4 Putting everything together: the functional view of metabolic regulation

From all the above, the functional view of metabolic regulation can be summarised as follows. Any functional representation of a biosynthetic pathway needs to include the demand for the end-product (compare Figs. 39 and 46).

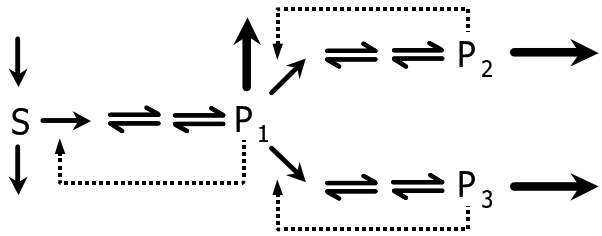

Figure 46: *The functional view of metabolic regulation.* The figure shows the same biosynthetic pathway as in Fig. 39, but now includes the demand reactions for the products P_1, P_2 and P_3, as indicated by wide arrows.

In contrast to the classical view of metabolic regulation as outlined on p. 79, the functional view rests on the following main tenets:

- the *demand processes control* the steady-state *flux*;
- the *non-equilibrium supply enzymes* maintain *homeostasis* in the concentrations of the end-products through allosteric end-product inhibition; and
- the *near-equilibrium supply enzymes* keep the intermediate *concentrations low* (preserving the solvent capacity of the cell). Because these enzymes are very active, they also allow the system to *respond rapidly* to changes in demand (e.g., low to high demand, or high to low demand) by reducing the time it takes to go from one steady state to another (the *transition time*).

11.5 Examples

At this stage the whole argument may appear to be a bit theoretical, so let us conclude by discussing a few real cellular pathways. The first thing you should realise is that the metabolic system in Figs. 42 and 44 is actually a representation of *serine biosynthesis,* with S standing for the glycolytic intermediate 3-phosphoglycerate, A for 3-phosphohydroxypyruvate, B for 3-phosphoserine and P for serine. Compare this with Fig. 24.44 on p. 768 in *Voet & Voet* or look up the pathway in any other biochemical textbook. The demand for serine includes protein biosynthesis, as well as other metabolic reactions requiring serine (e.g., glycine or cysteine biosynthesis). Serine biosynthesis is an interesting pathway, in that the feedback loop only exists in bacteria, whereas in plants and animals the feedback loop is absent and instead the third enzyme is inhibited uncompetitively by the product serine. Fig. 44 is thus a representation of bacterial serine biosynthesis, whereas Fig 42 is a representation of the pathway in plants and animals.

We thus find two regulatory motifs (Figs. 45(c) and (d)) in serine biosynthesis. Evidently, the kinetic regulation afforded by uncompetitive inhibition is sufficient in plants and animals, although allosteric feedback inhibition can in principle lead to better homeostasis in $\bar{p}$. In our own research, we have studied the regulation of the serine biosynthetic pathway in the bacterium *Escherichia coli,* with the view to performing an experimental supply-demand analysis.

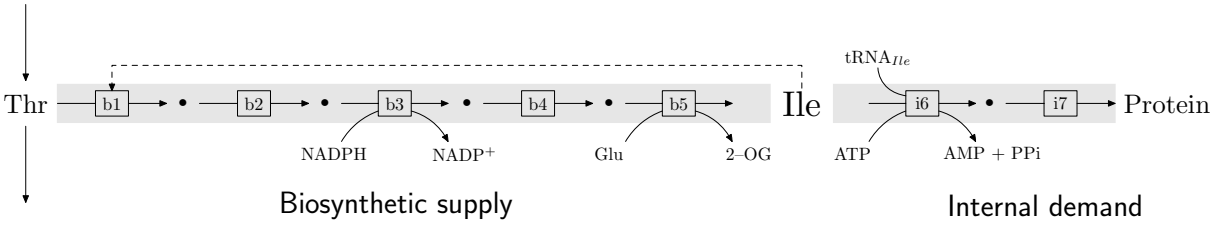

Figure 47: *Isoleucine biosynthesis as a supply-demand system.*

Fig. 47 shows the biosynthesis of isoleucine, another amino acid, from threonine. The same regulatory pattern is found here: the first enzyme in the biosynthetic pathway is inhibited allosterically by the product isoleucine. In the functional view, also here the demand controls the flux through the pathway, and the function of the feedback regulation is to maintain isoleucine concentrations homeostatically at a level far from equilibrium.

Finally, consider Fig. 48. The figure is a schematic representation of yeast glycolysis under anaerobic fermentative conditions. The glycolytic pathway is presented as a black box, and only the input and output metabolites are shown. When asked what is the function of glycolysis, many people will say the conversion of glucose to pyruvate or ethanol. However, the glycolytic pathway is central to *energy metabolism,* and therefore its most important function is the generation of ATP. In fact, for yeast or bacteria growing in the absence of oxygen, glycolysis is the *only* source of ATP, they cannot perform oxidative phosphorylation. When analysing glycolysis in supply-demand terms, it is therefore logical to include the *demand for ATP* as the demand process, with ATP and ADP (in fact, the ratio [ATP]/[ADP]) as the coupling intermediates. Applying our general insights from supply-demand analysis to the system in Fig. 48, we also expect here the *demand for ATP* to control the flux, with the glycolytic supply's function being the homeostatic maintenance of the [ATP]/[ADP] ratio. You will recall that at the beginning of the course we mentioned the studies that showed that the

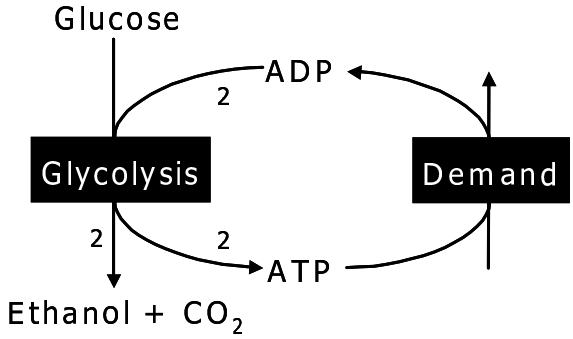

Figure 48: *Anaerobic yeast glycolysis as a supply-demand system.* Glycolysis converts one molecule of glucose to two molecules each of ethanol and carbon dioxide. In the process, two molecules of ATP are produced from ADP. The ATP demand constitutes all cellular processes requiring an input of metabolic free energy in terms of ATP.

enzymes of glycolysis do not control the glycolytic flux. Now you know why: in accordance with the functional view, flux-control does not lie in the glycolytic supply of ATP, but in the demand for ATP.

11.5.1 Biotechnological implications

Flux control by demand has an important caveat. Many biotechnological applications are based on the synthesis of certain end-products (e.g. amino acids) by microorganisms, and it is a central interest of biotech companies to maximise the production of these end-products. If the flux through the biosynthetic pathways for these end-products is controlled by the demand, any manipulation to increase production should focus on the *demand.* A manipulation on the supply side will be futile because the supply has no control over the flux. This explains the limited success of classical manipulation strategies, which have focussed primarily on altering supply enzyme properties (e.g., removing the feedback inhibition) or their cellular levels (e.g., over-expressing certain supply enzymes).

In contrast, an increase in the demand should result in an increase in the overall flux through the pathway. One way to achieve this is to introduce a leak for the end-product out of the cell. This could be a membrane protein allowing the product to leave the cell by facilitated diffusion, or it could be a transport protein that exports the product out of the cell at the expense of metabolic energy.

To close, let us return to the original problem posed in Section 10.1. For years, scientists have tried to increase the glycolytic flux by over-expressing the so-called "rate-limiting" step (phosphofructokinase or pyruvate kinase), but without success. With the background in this course, you can now understand why such an approach must necessarily fail. The problem is that there is absolutely no flux control in the supply: you can continue over-expressing supply enzymes until you are blue in the face, and the flux will simply not increase. Instead, Fig. 49 shows how it can be done. As explained above, we expect glycolytic flux control to lie in the demand for ATP. Hence, increasing this demand by adding an internal leak that hydrolyses ATP to ADP, should lead to an increased glycolytic flux (as indicted by the wide arrows in Fig. 49). In fact, recently scientists have over-expressed the F_1-ATPase in *Escherichia coli.* This protein forms part of the F_1F_0 ATP synthase complex, which synthesises ATP from the proton gradient generated during electron transport (see Voet & Voet, p. 586–590).

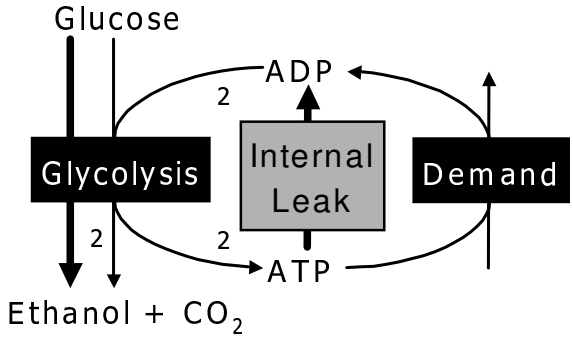

Figure 49: *Increasing glycolytic flux by introducing a leak for ATP.*

However, F_1 by itself without the F_0 part just hydrolyses ATP to ADP. In agreement with the prediction of supply-demand analysis, introducing such an internal "leak" into the bacteria increased their glycolytic flux significantly!

In general, when wanting to optimise production rates of biotechnological processes, supply-demand analysis leaves us with a clear message: because flux control is in the demand, you will have to **pull, not push**!