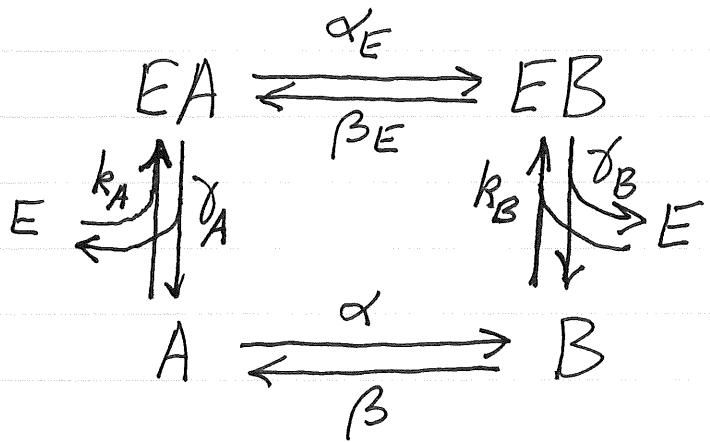


## Enzyme kinetics

A catalyst is a substance that speeds up a chemical reaction without being altered by the reaction. A biological macromolecule (usually a protein) that acts as a catalyst is called an enzyme.

Consider the following reaction scheme in which an enzyme E facilitates the reversible reaction  $A \rightleftharpoons B$ :



The differential equations for the concentrations of the five species  $A, B, EA, EB, E$  are as follows

/ 2

$$(1) \frac{d[A]}{dt} = -(\alpha + k_A[E])[A] + \beta[B] + \gamma_A[EA]$$

$$(2) \frac{d[B]}{dt} = -(\beta + k_B[E])[B] + \alpha[A] + \gamma_B[EB]$$

$$(3) \frac{d[EA]}{dt} = -(\alpha_E + \gamma_A)[EA] + \beta_E[EB] + k_A[E][A]$$

$$(4) \frac{d[EB]}{dt} = -(\beta_E + \gamma_B)[EB] + \alpha_E[EA] + k_B[E][B]$$

$$(5) \frac{d[E]}{dt} = -(k_A[A] + k_B[B])[E] \\ + \gamma_A[EA] + \gamma_B[EB]$$

Note that

$$(6) \frac{d}{dt} ([A] + [B] + [EA] + [EB]) = 0$$

$$(7) \frac{d}{dt} ([EA] + [EB] + [E]) = 0$$

Therefore, we have the following conserved quantities

$$(8) \quad [S^*] = [A] + [B] + [EA] + [EB]$$

$$(9) \quad [E^*] = [EA] + [EB] + [E]$$

Here  $[S^*]$  is the total concentration of substrate in the system, including all of its forms A, B, EA, EB; and  $[E^*]$  is the total concentration of enzyme in the system, including all of its forms EA, EB, E.

Since the system has a closed loop, and no energy source to drive a net flux around the loop, the rate constants must satisfy the principle of detailed balance

$$(10) \quad \alpha_E \gamma_B \beta k_A [E] = \beta_E \gamma_A \alpha k_B [E]$$

Note that  $[E]$  cancels out, and we are left with

$$(11) \quad \alpha_E \gamma_B \beta k_A = \beta_E \gamma_A \alpha k_B$$

Thus, the steady state of our system is one of thermodynamic equilibrium, in which every reaction is balanced by its own reverse reaction, and there is no net flux. Thus, at equilibrium

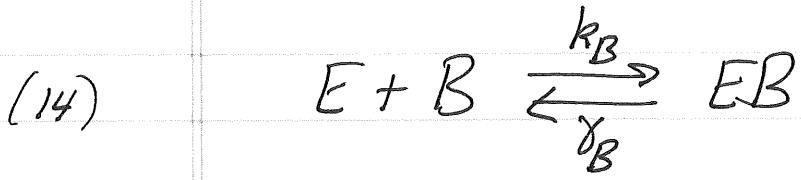
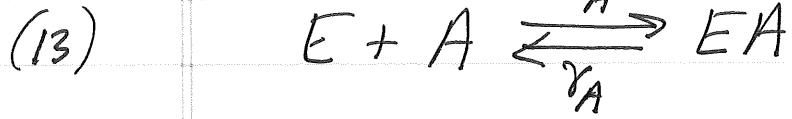
$$(12) \quad \alpha [A] = \beta [B]$$

exactly as if there were no enzyme present.

The ratio  $[A]/[B]$  ( $\sigma [B]/[A]$ ) at equilibrium is called the equilibrium constant of the reaction  $A \rightleftharpoons B$ ! We therefore reach the important conclusion that an enzyme cannot change the equilibrium constant of a reaction.

## Enzyme kinetics with fast and slow reactions

Suppose the reactions



are much faster than the reactions



Mathematically, we can model this situation by taking the following limits

$$(17) \quad \gamma_A \rightarrow \infty, k_A \rightarrow \infty \text{ with } \frac{\gamma_A}{k_A} = K_A$$

$$(18) \quad \gamma_B \rightarrow \infty, k_B \rightarrow \infty \text{ with } \frac{\gamma_B}{k_B} = K_B$$

6

Note that  $K_A$  and  $K_B$  have units of concentration.

If we had the reaction (13) in isolation, its equilibrium would be

$$(19) \quad k_A [E][A] = \gamma_A [EA]$$

or

$$(20) \quad \frac{[E][A]}{[EA]} = K_A$$

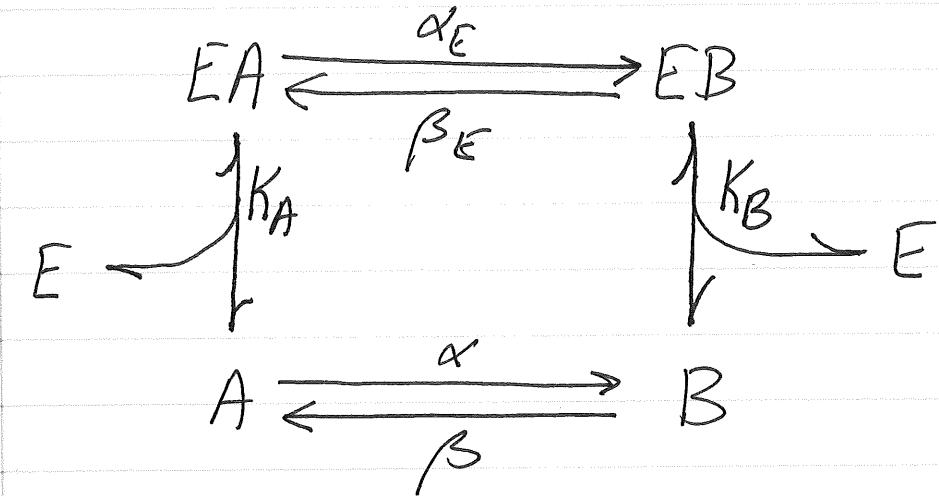
In the limit (17), equation (20) holds even when the reaction (13) is coupled to other reactions. Similarly, in the limit (18), we have

$$(21) \quad \frac{[E][B]}{[EB]} = K_B$$

Even though the reaction (14) is coupled to other reactions. The constants  $K_A$  and  $K_B$  are called the equilibrium constants (also known as dissociation constants) for the reactions (13) & (14), respectively.

17

The fast reactions are said to be in "rapid equilibrium" regardless of whether the system as a whole is in equilibrium or not. This is depicted as follows



Slow reactions are depicted by ordinary arrows with rate constants. Fast reactions are depicted by  $\rightleftharpoons$  and labeled by equilibrium constants. The equilibrium constants are written on the side of the arrow depicting dissociation.

Even though the fast reactions have infinite rates in each direction separately, they have finite net rates, although their net rates are unknown.

Let  $\rho_A$  be the net rate at which EA is dissociating into E + A, and let  $\rho_B$  be the net rate at which EB is dissociating into E + B. The variables  $\rho_A(t)$  and  $\rho_B(t)$  are unknown, and we will see later how they can be determined.

Now we can rewrite (1-5) in terms of  $\rho_A$  and  $\rho_B$ :

$$(22) \quad \frac{d[A]}{dt} = -\alpha[A] + \rho_A(t) + \beta[B]$$

$$(23) \quad \frac{d[B]}{dt} = -\beta[B] + \rho_B(t) + \alpha[A]$$

$$(24) \quad \frac{d[EA]}{dt} = -\alpha_E[EA] - \rho_A(t) + \beta_E[EB]$$

$$(25) \quad \frac{d[EB]}{dt} = -\beta_E[EB] - \rho_B(t) + \alpha_E[EA]$$

$$(26) \quad \frac{d[E]}{dt} = \rho_A(t) + \rho_B(t)$$

Equations (22-26) look somewhat simpler than (1-5), but they contain two additional unknowns,  $\rho_A(t)$  and  $\rho_B(t)$ .

The equations for these unknowns are (20-21), which we rewrite here as

$$(27) \quad [E][A] = K_A [EA]$$

$$(28) \quad [E][B] = K_B [EB]$$

We can differentiate these equations with respect to time, and then eliminate all of the time derivatives by making use of (22-26) as needed. This gives a pair of linear algebraic equations for  $\rho_A$  and  $\rho_B$ :

$$(29) \quad [E] (\rho_A - (\alpha[A] - \beta[B])) + (\rho_A + \rho_B)[A] \\ = -K_A (\rho_A + (\alpha_E[EA] - \beta_E[EB]))$$

$$(30) \quad [E] (\rho_B + (\alpha[A] - \beta[B])) + (\rho_A + \rho_B)[B] \\ = -K_B (\rho_B - (\alpha_E[EA] - \beta_E[EB]))$$

The above linear system can be rewritten as

$$(31) \quad \begin{pmatrix} ([E] + [A] + K_A) & [A] \\ [B] & ([E] + [B] + K_B) \end{pmatrix} \begin{pmatrix} P_A \\ P_B \end{pmatrix}$$

$$= \begin{pmatrix} [E](\alpha[A] - \beta[B]) - K_A(\alpha_E[EA] - \beta_E[EB]) \\ -[E](\alpha[A] - \beta[B]) + K_B(\alpha_E[EA] - \beta_E[EB]) \end{pmatrix}$$

Note that the matrix of this  $2 \times 2$  linear system is diagonally dominant (i.e., each diagonal term is strictly greater than the sum of the absolute values of the off-diagonal terms in the same row) and therefore cannot be singular.

Equation (31) implicitly defines  $P_A$  and  $P_B$  as functions of the state of the system, which is the list of concentrations

$[A]$ ,  $[B]$ ,  $[EA]$ ,  $[EB]$ ,  $[E]$ . In this way equations (22-26) together with (31) determine the rate of change of state when the state is known.

We can simplify the above system, however, in the following way. Recall the equilibrium relations (20) & (21). We are assuming that these hold at each instant whether or not the system as a whole is at equilibrium, since the corresponding reactions are fast. We write these relations as

$$(32) \quad [EA] = \frac{[A]}{K_A} [E], \quad [EB] = \frac{[B]}{K_B} [E]$$

Substitute these results into equation (9), and then solve for  $[E]$ ,  $[EA]$ , and  $[EB]$ . The results are

$$(33) \quad [E] = [E^*] \frac{1}{1 + [A]/K_A + [B]/K_B}$$

$$(34) \quad [EA] = [E^*] \frac{[A]/K_A}{1 + [A]/K_A + [B]/K_B}$$

$$(35) \quad [EB] = [E^*] \frac{[B]/K_B}{1 + [A]/K_A + [B]/K_B}$$

Equations (33-35) make it clear that the substrates A and B are competing for attention by the enzyme E.

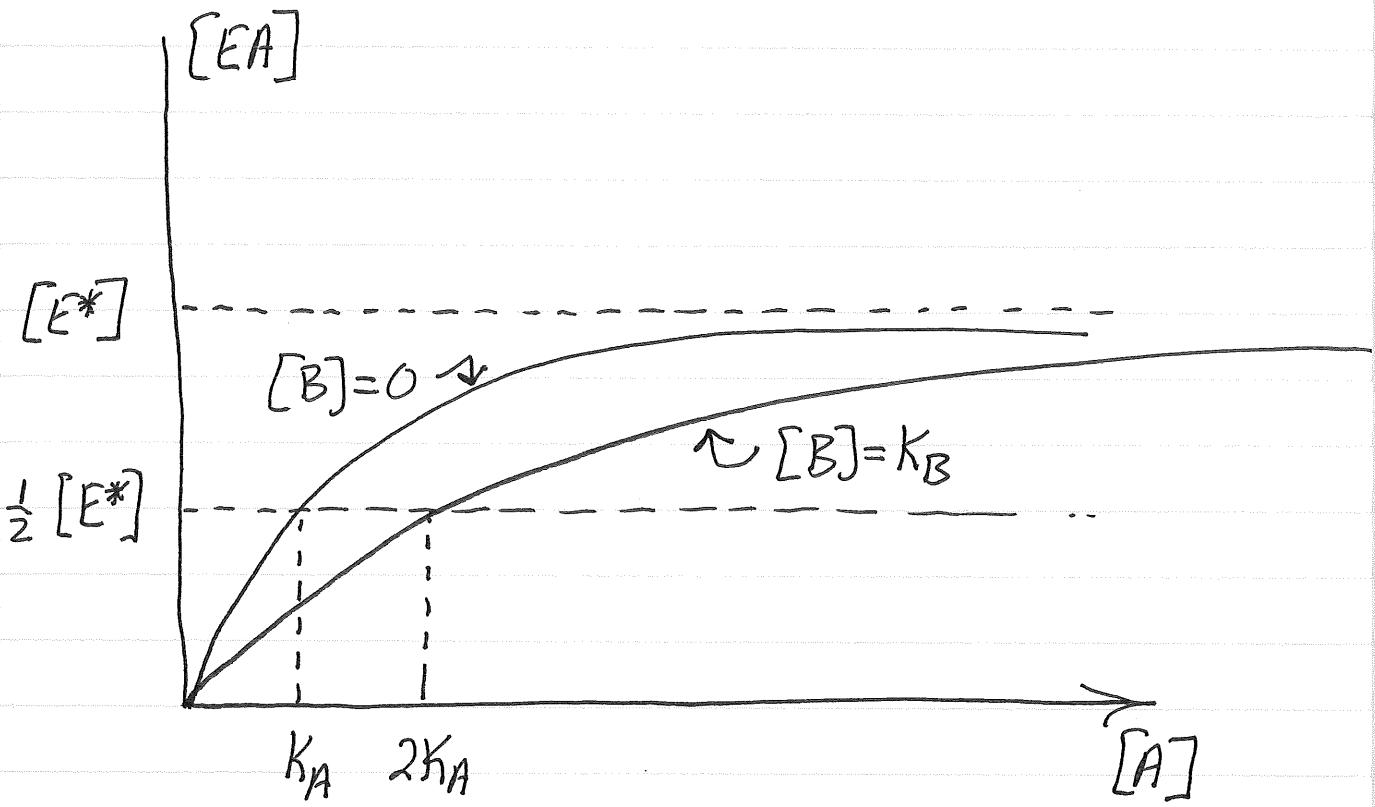
This can be made even more clear by dividing the numerators and denominators on the right-hand side of (34) by  $1 + \frac{[B]}{K_B}$ . This gives

$$(36) \quad \frac{[EA]}{[E^*]} = \frac{\frac{[A]}{K_A(1 + \frac{[B]}{K_B})}}{1 + \frac{[A]}{K_A(1 + \frac{[B]}{K_B})}}$$

Thus, the effect of  $[B]$  on the fraction of enzyme that is bound to A is to multiply the dissociation constant of A by the factor  $1 + [B]/K_B$ .

This is illustrated by the following plot:

13



Note that  $K_A$  and  $K_B$  set the relevant concentration scale of A and B, respectively.

Now we have only two unknowns  $[A]$  and  $[B]$  and we can get equations for them by adding (22) & (24), and similarly by adding (23) & (25). Note that in this way we eliminate  $p_A$  and  $p_B$ . We get

$$(37) \quad \frac{d}{dt} ([A] + [EA]) = -\alpha[A] - \alpha_E[EA] \\ + \beta[B] + \beta_E[EB]$$

$$(38) \quad \frac{d}{dt} ([B] + [EB]) = \alpha[A] + \alpha_E[EA] \\ - \beta[B] - \beta_E[\bar{E}B]$$

The right-hand sides of these equations are minus each other, as they should be, since  $[A] + [EA] + [B] + [EB]$  is a conserved quantity.

Now we can use (34-35) to rewrite these equations in terms of  $[A]$  and  $[B]$  alone.

First, consider the right-hand side of (38).  
It becomes

$$(39) \quad \alpha[A] - \beta[B] + [E^*] \frac{\left( \frac{\alpha_E[A]}{K_A} - \frac{\beta_E[B]}{K_B} \right)}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B}}$$

This can be simplified by making use of the principle of detailed balance. Dividing both sides of (11) by  $K_A K_B$  and recalling equations (17) & (18) that define  $K_A$  and  $K_B$ , we get

$$(40) \quad \alpha_E K_B \beta = \beta_E K_A \alpha$$

or

$$(41) \quad \frac{\alpha_E / K_A}{\beta_E / K_B} = \frac{\alpha}{\beta}$$

Equation (40) or (41) is a relationship between the four enzyme parameters  $K_A, K_B, \alpha_E, \beta_E$  and the two parameters  $\alpha, \beta$  that characterize the enzyme-free reaction. Thus, we can reduce the number of enzyme parameters to three.

A symmetrical way to do this is to introduce a constant  $K_E$  with units of concentration such that

$$(42) \quad \alpha_E K_E = \alpha K_A$$

$$(43) \quad \beta_E K_E = \beta K_B$$

The enzyme is now fully characterized by the three constants  $(K_A, K_B, K_E)$ , the rate constants  $\alpha_E$  and  $\beta_E$  can be obtained from (42-43), and detailed balance is assumed.

The expression (39) now becomes

$$(44) \quad (\alpha[A] - \beta[B]) \left( 1 + \frac{[E^*]/K_E}{1 + [A]/K_A + [B]/K_B} \right)$$

and we see once more that the presence of the enzyme has no effect on the ratio of  $[A]/[B]$  at equilibrium.

Equations (37-38) now become

$$(45) \quad -\frac{d}{dt} \left( [A] + [E^*] - \frac{[A]/K_A}{1 + [A]/K_A + [B]/K_B} \right)$$

$$= \frac{d}{dt} \left( [B] + [E^*] - \frac{[B]/K_B}{1 + [A]/K_A + [B]/K_B} \right)$$

$$= (\alpha[A] - \beta[B]) \left( 1 + \frac{[E^*]/K_E}{1 + [A]/K_A + [B]/K_B} \right)$$

We could now expand the time derivatives in (45) and get a pair of linear equations for  $d[A]/dt$  and  $d[B]/dt$  in terms of  $[A]$  and  $[B]$ . We will not follow this approach, however. Instead we define

$$(46) \quad [A^*] = [A] + [EA]$$

$$= [A] + [E^*] \frac{[A]/K_A}{1 + [A]/K_A + [B]/K_B}$$

$$(47) \quad [B^*] = [B] + [EB]$$

$$= [B] + [E^*] \frac{[B]/K_B}{1 + [A]/K_A + [B]/K_B}$$

Note that  $[A^*]$  and  $[B^*]$  are not conserved, although their sum is.

Now the time-derivative terms in (45) are just  $-d[A^*]/dt$  and  $d[B^*]/dt$ , so there is no difficulty about them, but instead we have the task of expressing  $[A]$  and  $[B]$  in terms of  $[A^*]$  and  $[B^*]$ .

Divide (46) by  $K_A$  and (47) by  $K_B$

let.

$$(48) \quad \theta_A = \frac{[A]}{K_A}, \quad \theta_A^* = \frac{[A^*]}{K_A}$$

$$(49) \quad \theta_B = \frac{[B]}{K_B}, \quad \theta_B^* = \frac{[B^*]}{K_B}$$

$$(50) \quad \lambda_A = \frac{[E^*]}{K_A}, \quad \lambda_B = \frac{[E^*]}{K_B}$$

Then (46) & (47) become

$$(51) \quad \theta_A^* = \theta_A + \lambda_A \frac{\theta_A}{1 + \theta_A + \theta_B}$$

$$(52) \quad \theta_B^* = \theta_B + \lambda_B \frac{\theta_B}{1 + \theta_A + \theta_B}$$

Our task is to find  $\theta_A$  and  $\theta_B$ ,

given  $\theta_A^*$ ,  $\theta_B^*$ ,  $\lambda_A$ , and  $\lambda_B$ .

Equations (51) & (52) can be rearranged as follows

$$(53) \quad \theta_A = \frac{\theta_A^*}{1 + \frac{\lambda_A}{1 + \theta_A + \theta_B}}$$

$$(54) \quad \theta_B = \frac{\theta_B^*}{1 + \frac{\lambda_B}{1 + \theta_A + \theta_B}}$$

Adding these equations and letting

$$(55) \quad \theta = \theta_A + \theta_B$$

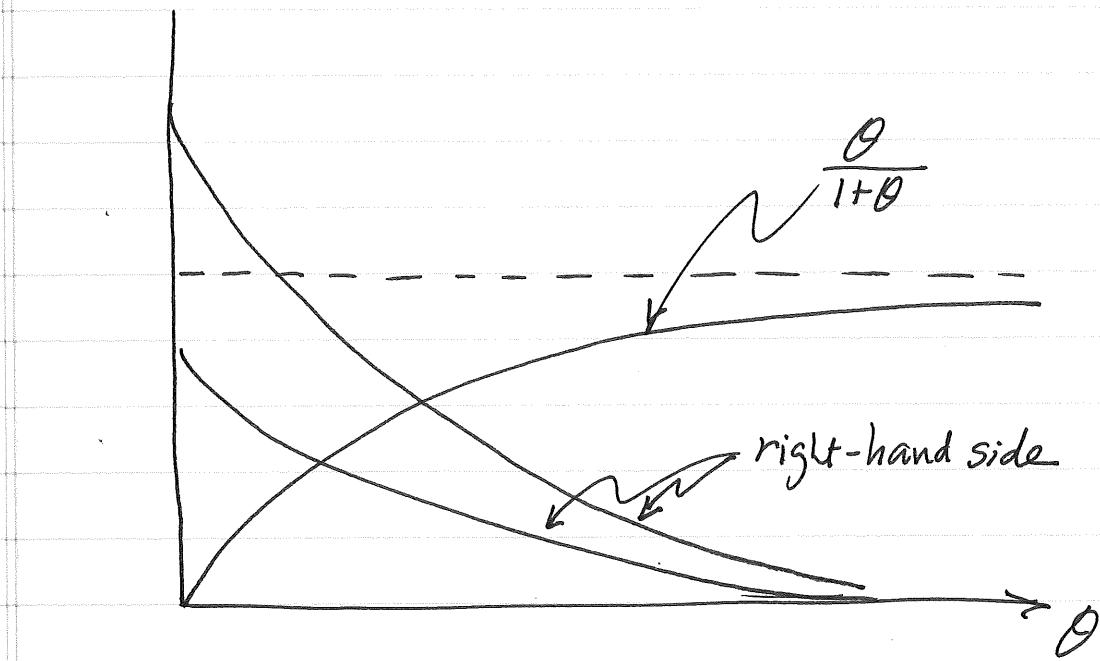
we get an equation for  $\theta$ :

$$(56) \quad \theta = \frac{\theta_A^{**}}{1 + \frac{d\theta}{1+\theta}} + \frac{\theta_B^{**}}{1 + \frac{d\theta}{1+\theta}}$$

Dividing both sides by  $1+\theta$ , we may rewrite this equation as

$$(57) \quad \frac{\theta}{1+\theta} = \frac{\theta_A^{**}}{1+\theta+d\lambda_A} + \frac{\theta_B^{**}}{1+\theta+d\lambda_B}$$

We are interested only in  $\theta \geq 0$ , and equation (57) obviously has exactly one solution with this property, since the left-hand side is a continuous function that increases monotonically from 0 to 1 as  $\theta$  goes from 0 to  $\infty$ , whereas the right-hand side is a continuous function that decreases monotonically from a positive value to zero as  $\theta$  goes from 0 to  $\infty$ . This is illustrated graphically as follows



The left-hand side of (57) is always  $\frac{\theta}{1+\theta}$ ,

but the right-hand side may vary depending on parameters. In all cases, however, there is exactly one solution on  $\theta \geq 0$ .

Equation (57) can be written as

$$(58) \quad f(\theta) = 0$$

where

$$(59) \quad f(\theta) = \frac{\theta}{1+\theta} - \frac{\theta_A^*}{1+\theta+\lambda_A} - \frac{\theta_B^*}{1+\theta+\lambda_B}$$

$$(60) \quad f'(\theta) = \frac{1}{(1+\theta)^2} + \frac{\theta_A^*}{(1+\theta+\lambda_A)^2} + \frac{\theta_B^*}{(1+\theta+\lambda_B)^2}$$

These formulae make it straightforward to solve (58) by Newton's method.

Some other possibilities are to use the Matlab function `fzero`, or to rewrite (58) as a cubic equation and then use the known (but complicated!) formula for the solution of a cubic equation, or the Matlab function `roots`, keeping in mind that we are only interested in a solution that is real and positive (and we know in advance that there will be exactly one of these).

Thus, if we are given  $[A^*]$  and  $[B^*]$  at some particular time, we can find  $[A]$  and  $[B]$  at that time as follows. Recall that  $[E^*]$ ,  $K_A$ ,  $K_B$  are given constants, and that  $\alpha_A$  and  $\alpha_B$  are therefore known, see (53).

First, evaluate  $\theta_A^* = [A^*]/K_A$  and  $\theta_B^* = [B^*]/K_B$ . Then solve equation (58) for  $\theta$ , and recall that  $\theta = \theta_A + \theta_B$ . With  $\theta_A + \theta_B$  known, use (53) & (54) to evaluate  $\theta_A$  and  $\theta_B$  separately (and check that they add up to the value of  $\theta$  that was found on solving (58)!) Finally, set  $[A] = K_A \theta_A$  and  $[B] = K_B \theta_B$ , see (48-49).

With  $[A]$  and  $[B]$  known, we can evaluate the expression (44), which is equal to  $-d[A^*]/dt$  and  $d[B^*]/dt$ .

The above procedure defines  $d[A^*]/dt$  and  $d[B^*]/dt$  as functions of  $[A^*]$  and  $[B^*]$ , so  $[A^*]$  and  $[B^*]$  are suitable state variables of our system, and in fact we can work with just one of them, since their sum is constant.

The expression (44) for the net rate of conversion from any form of A to any form of B can be written as a sum of two terms

$$(61) \quad \left( \alpha[A] - \beta[B] \right) + \left( \frac{[E^*]}{K_E} \left( \frac{\alpha[A] - \beta[B]}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B}} \right) \right)$$

The first term accounts for reactions that happen without the intervention of the enzyme, i.e., for reactions of the type  $A \rightleftharpoons B$ , and the second term accounts for reactions that happen on the enzyme itself, i.e., reactions of the type  $EA \rightleftharpoons EB$ . It is a consequence of the principle of detailed balance that the second term also involves the factor  $\alpha[A] - \beta[B]$ .

If we divide the second term by  $[E^*]$ , we get the net number of reactions of the type  $EA \rightleftharpoons EB$  that occurs per unit time per molecule of the enzyme. This will be denoted  $v([A], [B])$ . Thus

$$(62) \quad v([A], [B]) = \frac{1}{K_E} \frac{\alpha[A] - \beta[B]}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B}}$$

Note that

$$(63) \quad v([A], 0) = v_A^{\max} \frac{[A]/K_A}{1 + [A]/K_A}$$

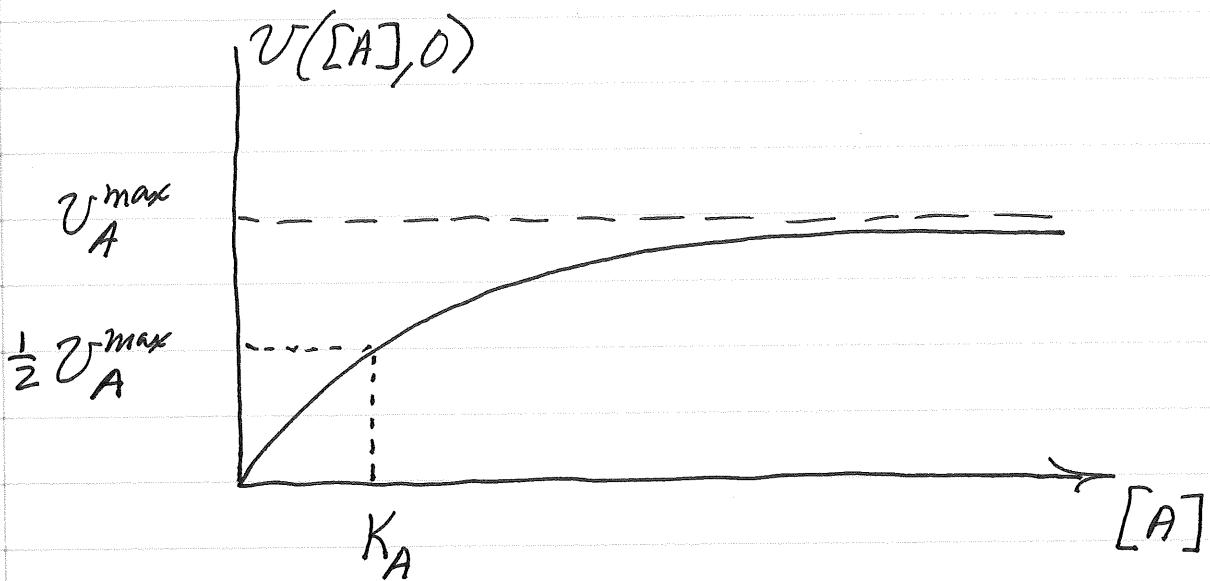
$$(64) \quad -v(0, [B]) = v_B^{\max} \frac{[B]/K_B}{1 + [B]/K_B}$$

where

$$(65) \quad v_A^{\max} = \frac{\alpha K_A}{K_E} = \alpha_E$$

$$(66) \quad v_B^{\max} = \frac{\beta K_B}{K_E} = \beta_E$$

Equations (63) & (64) describe what is called Michaelis-Menten enzyme kinetics in which the reaction rate depends on the substrate concentration as shown (for  $[A]$ ) in the following plot.



Note, however, that equations (63) & (64) are only applicable to the simple situation in which one of the two species is present at negligible concentration. This neglects not only the back reaction but also the effect of the product on the forward reaction that comes about because of its competition for the active site on the enzyme. (See equation (36) and the related discussion and plot.)

It should be noted that the above derivation of Michaelis-Menten kinetics is not the usual one. A limiting case that is not the same as (17-18) but leads to similar results is as follows.\*

Suppose that the non-enzymatic reaction is very slow, and that the concentration of enzyme in the system is small, but not very small. These statements can be formalized by introducing a small parameter  $\varepsilon$  and letting

$$(67) \quad \alpha = \varepsilon^2 \bar{\alpha}, \quad \beta = \varepsilon^2 \bar{\beta}, \quad [E^*] = \varepsilon \overline{[E^*]}$$

We expect, then, that the concentrations of the various forms of the enzyme will be  $O(\varepsilon)$ , so we write

$$(68) \quad [E] = \varepsilon \overline{[E]}, \quad [EA] = \varepsilon \overline{[EA]}, \quad [EB] = \varepsilon \overline{[EB]}$$

With these changes of variables, equations (1-5) become

\*Lin CC & Segel LA: Mathematics Applied to Deterministic Problems in the Natural Sciences  
New York: Macmillan 1974, Chapter 10

$$(69) \quad \frac{d[A]}{dt} = -(\varepsilon^2 \bar{\alpha} + \varepsilon k_A \overline{[E]}) [A] \\ + \varepsilon^2 \bar{\beta} [B] + \varepsilon \gamma_A \overline{[EA]}$$

$$(70) \quad \frac{d[B]}{dt} = -(\varepsilon^2 \bar{\beta} + \varepsilon k_B \overline{[E]}) [B] \\ + \varepsilon^2 \bar{\alpha} [A] + \varepsilon \gamma_B \overline{[EB]}$$

$$(71) \quad \frac{d\overline{[EA]}}{dt} = -(\alpha_E + \gamma_A) \overline{[EA]} + \beta_E \overline{[EB]} + k_A \overline{[E]} [A]$$

$$(72) \quad \frac{d\overline{[EB]}}{dt} = -(\beta_E + \gamma_B) \overline{[EB]} + \alpha_E \overline{[EA]} + k_B \overline{[E]} [B]$$

$$(73) \quad \frac{d\overline{[E]}}{dt} = - (k_A [A] + k_B [B]) \overline{[E]} \\ + \gamma_A \overline{[EA]} + \gamma_B \overline{[EB]}$$

Note that  $\varepsilon$  does not appear in equations (71-73) because it has canceled out.

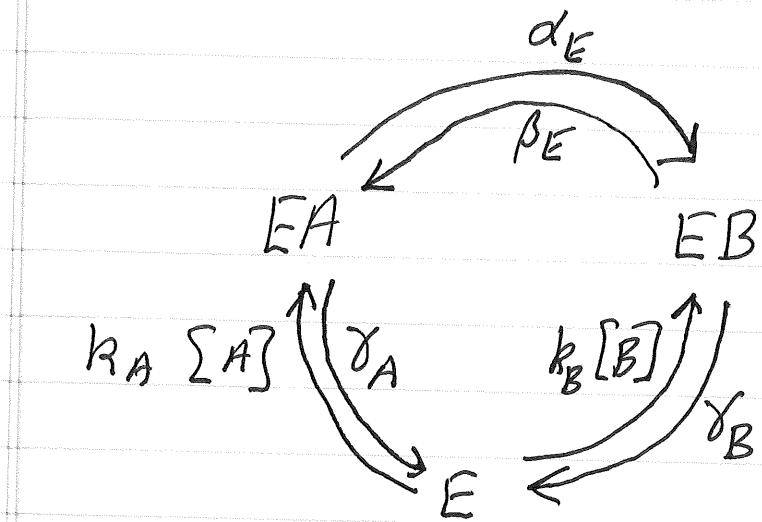
The plan now is to let  $\varepsilon \rightarrow 0$  in equations (69-73). There are two different time scales of interest.

The faster time scale equations are obtained by letting  $\varepsilon \rightarrow 0$  without making any further change of variables. In equations (69-70), this gives

$$(74) \quad \frac{d[A]}{dt} = 0, \quad \frac{d[B]}{dt} = 0$$

Equations (71-73) are unchanged, since they do not involve  $\varepsilon$ , but because of (74) we have the simplification that  $[A]$  and  $[B]$  in equations (71-73) are constant.

Equations (71-73) with  $[A]$  and  $[B]$  constant are the equations of the following reaction scheme



Here, since we are regarding  $[A]$  and  $[B]$  as parameters, we do not show explicitly the entry of A in the reaction  $E+A \rightarrow EA$  or the exit of A from  $EA \rightarrow E+A$ , and similarly for B. Note, however, that each clockwise turn of the above loop converts one molecule of A into one molecule of B, and that each counterclockwise turn converts one molecule of B into one molecule of A.

with  $[A]$  and  $[B]$  constant

32

Equations (71-73) are a linear system of ODE in the unknowns  $\overline{[E]}$ ,  $\overline{[EA]}$ ,  $\overline{[EB]}$ . This system has the conserved quantity

$$(75) \quad \overline{[E^*]} = \overline{[E]} + \overline{[EA]} + \overline{[EB]}$$

So we can regard it as a system with two unknowns, e.g.,  $\overline{[EA]}$  and  $\overline{[EB]}$ , and drop one of the equations.

Although we do not prove it here, all solutions exponentially approach the steady state given by

$$(76) \quad 0 = -(\alpha_E + \gamma_A) \overline{[EA]} + \beta_E \overline{[EB]} \\ + k_P [A] (\overline{[E^*]} - \overline{[EA]} - \overline{[EB]})$$

$$(77) \quad 0 = -(\beta_E + \gamma_B) \overline{[EB]} + \alpha_E \overline{[EA]} \\ + k_B [B] (\overline{[E^*]} - \overline{[EA]} - \overline{[EB]})$$

This pair of equations can be written as

$$(78) \quad \begin{pmatrix} \alpha_E + \gamma_A + k_A[A] & k_A[A] - \beta_E \\ k_B[B] - \alpha_E & \beta_E + \gamma_B + k_B[B] \end{pmatrix} \begin{pmatrix} \overline{[EA]} \\ \overline{[EB]} \end{pmatrix} = \overline{[E^*]} \begin{pmatrix} k_A[A] \\ k_B[B] \end{pmatrix}$$

and the solution is

$$(79) \quad \overline{[EA]} = \overline{[E^*]} \frac{(\beta_E + \gamma_B)k_A[A] + \beta_E k_B[B]}{\Delta}$$

$$(80) \quad \overline{[EB]} = \overline{[E^*]} \frac{\alpha_E k_A[A] + (\alpha_E + \gamma_A)k_B[B]}{\Delta}$$

where  $\Delta$  is the determinant of the matrix on the left-hand side of (78), that is

$$(81) \quad \begin{aligned} \Delta &= (\alpha_E + \gamma_A + k_A[A])(\beta_E + \gamma_B + k_B[B]) \\ &\quad - (k_A[A] - \beta_E)(k_B[B] - \alpha_E) \\ &= \alpha_E \gamma_B + \beta_E \gamma_A + \gamma_A \gamma_B \\ &\quad + (\alpha_E + \beta_E + \gamma_B) k_A[A] \\ &\quad + (\alpha_E + \beta_E + \gamma_A) k_B[B] \end{aligned}$$

For completeness, we can also evaluate  $\overline{[E]}$  from (75). It is given by

$$(82) \quad \overline{[E]} = \overline{[E^*]} \frac{\alpha_E \gamma_B + \beta_E \gamma_A + \gamma_A \gamma_B}{\Delta}$$

Now the steady-state flux around the loop of reactions involving the species E, EA, EB can be evaluated from any one of the three reactions in the loop, together with its reverse reaction. We leave it to the reader to check that the result is the same regardless of which reaction is chosen.

We use

$$(83) \quad \bar{f} = \alpha_E \overline{[EA]} - \beta_E \overline{[EB]} \\ = \frac{\alpha_E \gamma_B k_A [A] - \beta_E \gamma_A k_B [B]}{\Delta}$$

Now divide the numerators and denominators by  $(\gamma_A \gamma_B)$ , and recall the definitions

$$(84) \quad K_A = \frac{\gamma_A}{k_A}, \quad K_B = \frac{\gamma_B}{k_B}$$

In this way, we get the result

$$(85) \quad \bar{f} = \overline{[E^*]} - \frac{\alpha_E \frac{[A]}{K_A} - \beta_E \frac{[B]}{K_B}}{\left( \left( 1 + \frac{\alpha_E}{\gamma_A} + \frac{\beta_E}{\gamma_B} \right) + \left( 1 + \frac{\alpha_E + \beta_E}{\gamma_B} \right) \frac{[A]}{K_A} + \left( 1 + \frac{\alpha_E + \beta_E}{\gamma_A} \right) \frac{[B]}{K_B} \right)}$$

This expression is remarkably similar to the enzymatic term in (39). In fact, it reduces to that term if we now take the limit described in (17-18).

This completes the analysis of the faster time scale, on which  $[A]$  and  $[B]$  are changing so slowly that they can be regarded as constants.

To capture the dynamics of  $[A]$  and  $[B]$ , we introduce a new time variable

$$(86) \quad \tau = \varepsilon t$$

Corresponding to any function  $\phi(t)$ , there is a function  $\Phi(\tau)$  related to  $\phi(t)$  by

$$(87) \quad \Phi(\tau) = \phi(t)$$

whenever  $\tau$  and  $t$  are related by (86).

Note that  $\Phi$  is not the same function as  $\phi$ , even though these two functions take on the same values at corresponding points. To see this more clearly, we can substitute (86) into (87) and get either of the two formulae

$$(88) \quad \Phi(\tau) = \phi\left(\frac{\tau}{\varepsilon}\right), \quad \Phi(\varepsilon t) = \phi(t)$$

the first of which defines  $\Phi$  when  $\phi$  is given, and the second of which defines  $\phi$  in terms of  $\Phi$ .

Now we can differentiate with respect to  $\tau$  in the first version of (88) or with respect to  $t$  in the second version:

$$(89) \quad \frac{d\Phi}{d\tau}(\tau) = \frac{1}{\varepsilon} \frac{d\phi}{dt}\left(\frac{\tau}{\varepsilon}\right), \quad \varepsilon \frac{d\Phi}{d\tau}(\varepsilon t) = \frac{d\phi}{dt}(t)$$

Both of these equations are equivalent to

$$(90) \quad \varepsilon \frac{d\Phi}{d\tau}(\tau) = \frac{d\phi}{dt}(t)$$

in which it is understood, as in (87) that  $\tau$  and  $t$  are ~~set~~ such that  $\tau = \varepsilon t$ .

Note that (90) is easy to remember if we think in terms of differentials. It is true because  $d\Phi = d\phi$  and  $d\tau = \varepsilon dt$ .

Once we understand how to transform the time derivatives, it is no longer so important to use different names for a function of  $t$  and its corresponding function of  $\tau$ , so we will not bother to do so.

Thus, to get the slower time-scale version of equations (69-73), we write

$$(91) \quad \frac{d[A]}{dt} = \varepsilon \frac{d[A]}{d\tau}$$

and do the same for all of the other time derivatives. Then we divide both sides by  $\varepsilon$  in (69-70), and finally we let  $\varepsilon \rightarrow 0$  in all of the equations. The results are

$$(92) \quad \frac{d[A]}{dt} = -k_A \overline{[E]} [A] + \gamma_A \overline{[EA]}$$

$$(93) \quad \frac{d[B]}{dt} = -k_B \overline{[E]} [B] + \gamma_B \overline{[EB]}$$

$$(94) \quad 0 = -(\alpha_E + \gamma_A) \overline{[EA]} + \beta_E \overline{[EB]} + k_A \overline{[E]} [A]$$

$$(95) \quad 0 = -(\beta_E + \gamma_B) \overline{[EB]} + \alpha_E \overline{[EA]} + k_B \overline{[E]} [B]$$

$$(96) \quad 0 = - (k_A [A] + k_B [B]) \overline{[E]} \\ + \gamma_A \overline{[EA]} + \gamma_B \overline{[EB]}$$

Note that (94-96) are just the steady-state equations corresponding to (71-73). We have already solved these steady-state equations, see (79-85). Here we see that the limit  $t \rightarrow \infty$  in the faster time-scale equations holds at each  $\tau$  in the slower time-scale equations. This is called a "quasi-steady state," since there is a different such state for each  $[A]$ ,  $[B]$ , and  $[A]$ ,  $[B]$  are slowly changing, as described by (92-93).

By combining (92) & (94), we get

$$(97) \quad \frac{d[A]}{dt} = -(\alpha_E \overline{[EA]} - \beta_E \overline{[EB]}) = -\bar{f}$$

and similarly, by combining (93) & (95),

$$(98) \quad \frac{d[B]}{dt} = \alpha_E \overline{[EA]} - \beta_E \overline{[EB]} = +\bar{f}$$

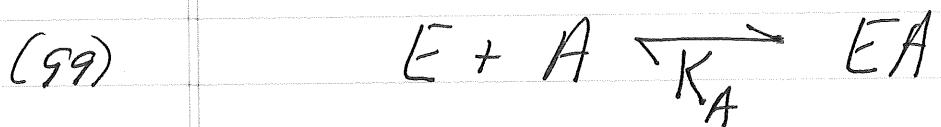
where  $\bar{f}$  is given by (85). This completes the description of the slower time scale.

Remark : The non-enzymatic reaction does not appear in equations (97-98) because we made the assumption that it is "very slow", i.e., that its rates are  $O(\epsilon^2)$ , so that the enzymatic version of the reaction dominates the picture even though the concentration of the enzyme is  $O(\epsilon)$ .

This was not essential. We could have made the rates of the non-enzymatic reaction  $O(\epsilon)$ , and then they would have appeared in (97-98). In many cases however, it is realistic to neglect the non-enzymatic reaction.

In summary, we have described two different limiting cases of enzyme kinetics with remarkably similar but not identical results.

In the first case, we assumed that the reactions



are in rapid equilibrium, as indicated

by the notation  $\xrightleftharpoons{K}$  with a double-headed arrow labeled by the equilibrium constant of the reaction. This means that the individual reactions are so fast that the concentrations adjust as if the system

were at equilibrium, even though it may not actually be at equilibrium. In particular, this means that we may use the equations

$$(101) \quad K_A = \frac{[E][A]}{[EA]}, \quad K_B = \frac{[E][B]}{[EB]}$$

together with the other equations of the system, to determine the net rates at which the reactions (99-100) are occurring. These net rates are finite, and we have shown how they can be determined, see equations (22-31).

Because of the rapid equilibria (99-100), the natural variables to use in describing the state of the system are

$$(102) \quad [A^*] = [A] + [EA], \quad [B^*] = [B] + [EB]$$

When we use these variables, however, we need to be able to solve for  $[A]$ ,  $[B]$ , and therefore also  $[EA]$ ,  $[EB]$ , with given values of  $[A^*]$ ,  $[B^*]$ , and of course also a given value of the constant

$[E^*] = [E] + [EA] + [EB]$ . We have shown how this can be done, see equations (46-60).

In the second case the key assumption is that the enzyme concentration is small in comparison to the substrate concentration. This leads to a quasi-steady state involving the species  $EA$ ,  $EB$ ,  $E$  which are driven around a cycle by the free concentrations of  $A$  and  $B$ , which are changing so slowly that they may be regarded as constant. Each turn of the cycle in one direction converts a molecule of  $A$  into a molecule of  $B$ , and each turn in the other direction does the opposite. The net flux around this cycle is given by (85), which therefore determines the rate of conversion of  $A$  to  $B$ , or vice versa, see (97-98). Because this theory is based on the assumption that the enzyme concentration is small in comparison to that of the substrate, there is no need to distinguish  $[A]$  from  $[A^*]$  or  $[B]$  from  $[B^*]$ , and hence no need to solve for  $[A]$  and  $[B]$  gives  $[A^*]$  and  $[B^*]$ .

The two limiting cases that we have just described are not inconsistent. It is perfectly possible for the enzyme to be present in low concentration and for the binding/unbinding rates of substrate to/from the enzyme to be fast. When both of these conditions are satisfied, everything is as simple as possible. In particular, because the concentration of the enzyme is small, we may set  $[A^*] = [A]$  and  $[B^*] = [B]$ . Also, because the rates of binding and unbinding are fast, we get a simpler formula for the flux around the quasi-steady state, and hence for the rate of conversion from A to B or from B to A, as has already been noted following equation (85).

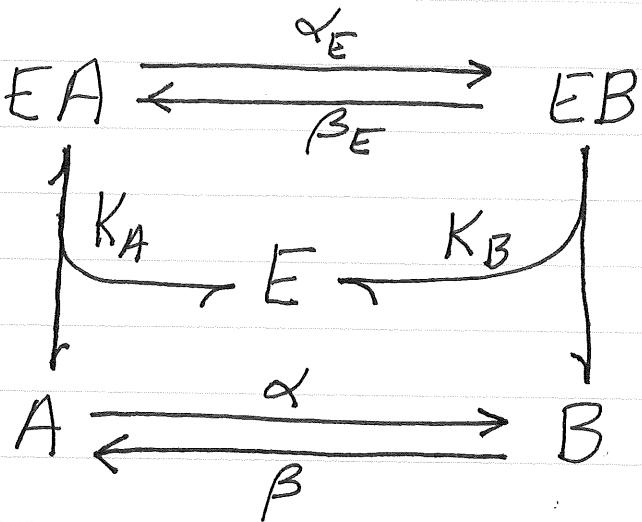
Of course, it is also possible that neither of the above limiting cases is applicable, and then we should just use equations (1-5). An interesting project would be to compare the behavior of the full system (1-5) to the behavior predicted by either or both of the above limiting cases for various choices of parameters.

## Event-driven simulations of enzyme kinetics involving rapid equilibria

Now we consider stochastic simulations in which integer numbers of molecules are tracked and updated whenever a chemical reaction occurs. Methodology for such simulations has been introduced in an earlier section of these notes.

When some of the reactions have much higher rate constants than others, a practical difficulty arises that most of the events that occur involve the fast reactions, so a lot of computation is involved in getting from one slow event to the next slow event. One way to avoid this difficulty is to regard the fast reactions as being "at equilibrium" and to simulate explicitly only the events corresponding to the slow reactions. This requires, however, a microscopic analysis of the equilibrium state, as we now discuss.

Consider again the enzymatic reaction scheme



Here we draw E on the inside to emphasize that we are actually dealing with one rapid equilibrium involving all five of the molecular species shown above.

Let  $V$  be the volume of the chamber in which the above reactions are happening.

The parameters  $\alpha, \beta, \alpha_E, \beta_E$

have units of  $1/\text{time}$ , and each of them can be interpreted as a probability per unit time. For example,

$\alpha$  is the probability per unit time that a particular free molecule of A will become a free molecule of B.

The parameters  $K_A$  and  $K_B$  are ratios of fast rate constants:

$$(103) \quad K_A = \frac{\gamma_A}{k_A}, \quad K_B = \frac{\gamma_B}{k_B}$$

Here  $\gamma_A$  and  $\gamma_B$  have units of 1/time and can be interpreted as probabilities per unit time, but  $k_A$  and  $k_B$  have units of volume/time. As we have discussed previously, the probabilities per unit time corresponding to  $k_A$  and  $k_B$  are

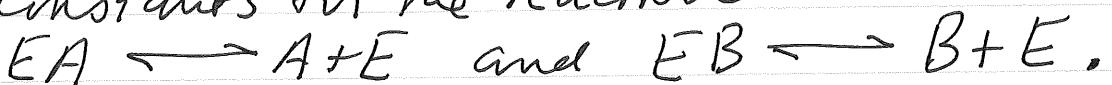
$$(104) \quad r_A = \frac{k_A}{V}, \quad r_B = \frac{k_B}{V}$$

The parameter  $r_A$  is the probability per unit time that a particular free molecule of A combines with a particular free molecule of E to form EA, and  $r_B$  is the corresponding probability per unit time for B.

It is useful to define

$$(105) \quad R_A = \frac{\gamma_A}{r_A} = K_A V, \quad R_B = \frac{\gamma_B}{r_B} = K_B V$$

Note that  $R_A$  and  $R_B$  are dimensionless, and volume-dependent. They may be thought of as the microscopic equilibrium constants for the reactions



(It is important here that we express concentration as the number of molecules per unit volume, not as the number of moles per unit volume.)

In stochastic simulation of the above system, the state of the system at any given time is described by the integers

$$(106) \quad n_{A^*} = (\text{number of molecules of } A) + (\text{number of molecules of } EA)$$

$$(107) \quad n_{B^*} = (\text{number of molecules of } B) + (\text{number of molecules of } EB)$$

These change in steps of  $\pm 1$  when any one of the finite-rate reactions (i.e., the reactions with rate constants  $\alpha, \beta, \alpha_E, \beta_E$ ) occurs, and their sum is constant.

An important parameter is the <sup>constant</sup> integer

$$(108) \quad n_{E^*} = (\text{number of molecules of } E) + (\text{number of molecules of } EA) + (\text{number of molecules of } EB)$$

The events of the simulation will be the occurrences of any one of the finite-rate reactions.

Between events, we assume that the system is in the state of equilibrium that it would reach if the finite-rate reactions could not occur at all. This state of equilibrium depends on the values of  $n_{A^*}$  and  $n_{B^*}$ , which are constant between events.

The following table lists the reactions that can occur, and for each reaction gives its probability per unit time and also the change that it makes in the state of the system.

Reaction	Probability/time	$\Delta n_{A^*}$	$\Delta n_{B^*}$
$A \rightarrow B$	$\bar{n}_A \alpha$	-1	+1
$B \rightarrow A$	$\bar{n}_B \beta$	+1	-1
$EA \rightarrow EB$	$\bar{n}_{EA} \alpha_E$	-1	+1
$EB \rightarrow EA$	$\bar{n}_{EB} \beta_E$	+1	-1

In this table,  $\bar{n}_A$ ,  $\bar{n}_B$ ,  $\bar{n}_{EA}$ ,  $\bar{n}_{EB}$  are the mean number of molecules of the species A, B, EA, EB respectively.

These mean values have to be computed from the probability distribution associated with the state of equilibrium

that we are assuming exists between events.  
 This is a different state of equilibrium for each pair  $(n_{A^*}, n_{B^*})$  so the variables  $\bar{n}_A, \bar{n}_B, \bar{n}_{EA}, \bar{n}_{EB}$  are functions of  $(n_{A^*}, n_{B^*})$ . How these functions are defined and evaluated will be discussed next.

Let

(109)  $P(i, j | a, b, e) = \text{Probability that } n_{EA} = i \text{ and } n_{EB} = j, \text{ in a state of equilibrium for which } n_{A^*} = a, n_{B^*} = b, n_{E^*} = e.$

Here  $a, b, e$  are fixed non-negative integers, and  $i, j$  are non-negative integers that satisfy all of the following

(110)  $i \leq a, j \leq b, i+j \leq e$

The equations that govern  $P(i, j | a, b, e)$   
are as follows

$$(111) \quad \gamma_A(i+1) P(i+1, j | a, b, e)$$

$$= r_A(a-i)(e-(i+j)) P(i, j | a, b, e)$$

$$(112) \quad \gamma_B(j+1) P(i, j+1 | a, b, e)$$

$$= r_B(b-j)(e-(i+j)) P(i, j | a, b, e)$$

In (111) we have balanced the number of occurrences per unit time of  $i \rightarrow i+1$  against the number of occurrences per unit time of  $i \rightarrow i+1$ , and similarly for  $j$  in (112).

Equations (111) & (112) can also be written in terms of the microscopic equilibrium constants  $R_A$  and  $R_B$  as follows:

$$(113) \quad P(i+1, j | a, b, e)$$

$$= \frac{L}{R_A} \frac{(a-i)(e-(i+j))}{(i+1)} P(i, j | a, b, e)$$

$$(114) \quad P(i, j+1 | a, b, e)$$

$$= \frac{1}{R_B} \frac{(b-j)(e-(i+j))}{(j+1)} P(i, j | a, b, e)$$

From these two formulae, we can express  $P(i+1, j+1 | a, b, e)$  in terms of  $P(i, j | a, b, e)$  in two different ways. The first way is to raise  $j$  by 1 in (113) and then substitute (114) into the resulting equation. The second way is to raise  $i$  by 1 in (114) and then substitute (113) into the resulting equation. The reader should verify that the result is the same either way.

This path independence is a consequence of the principle of detailed balance, applied here on a microscopic level.

Because of the above path-independence, we can evaluate  $P(i,j|a,b,e)$  in terms of  $P(0,0|a,b,e)$  by following any path from  $(0,0)$  to  $(i,j)$  on the lattice of pairs of integers. This will be exploited in the following Matlab function that evaluates  $P$  and then computes from  $P$  the mean values that are needed for event-driven simulation.

Because Matlab array indices always start from 1, we store  $P(i,j|a,b,e)$  in an array  $p$  at location  $(1+i, 1+j)$ .

For simplicity, we evaluate  $P$  for  $0 \leq i \leq a$  and  $0 \leq j \leq b$ , without regard to the inequality  $i+j \leq e$ . When  $i+j > e$ , however, the value of  $P$  will be zero. This could be inefficient, especially if  $a$  and/or  $b$  are large but  $e$  is small.

function [nAbar, nBbar, nEAbar, nEBbar] = ...  
 rapid\_equilibrium (nAstar, nBstar, nEstar, RA, RB)

$$a = nAstar$$

$$b = nBstar$$

$$e = nEstar$$

$$p = zeros(1+a, 1+b)$$

$$p(1+(0), 1+(0)) = 1$$

for  $i = 0 : (a-1)$

$$p(1+(i+1), 1+(0)) = \dots$$

$$\frac{p(1+(i), 1+(0)) * (a-i) * (e-i)}{(RA * (i+1))} \dots$$

end

$i = 0 : a$  % note implied loop over  $i$  in the following

for  $j = 0 : (b-1)$

$$p(1+(i), 1+(j+1)) = \dots$$

$$\frac{(p(1+(i), 1+(j)) * (e-i-j)) * (b-j)}{(RB * (j+1))} \dots$$

end

$$p = p / sum(sum(p)) \quad \% \text{ normalization.}$$

$$psum1 = sum(p, 1)$$

$$psum2 = sum(p, 2)$$

$$nEAbar = sum((0:a) .* psum2)$$

$$nEBbar = sum((0:b) .* psum1)$$

$$nAbar = nAstar - nEAbar$$

$$nBbar = nBstar - nEBbar$$

Alternatively, we can write a formula for  $P(i,j|a,b,e)$  in terms of factorials.

Repeated use of (113) with  $j=0$  gives

$$(115) \quad P(i,0|a,b,e) =$$

$$\frac{1}{R_A^i} \frac{a!}{(a-i)!} \frac{e!}{(e-i)!} \frac{1}{i!} P(0,0|a,b,e)$$

Similarly, repeated use of (114) with any fixed value of  $i$  gives

$$(116) \quad P(i,j|a,b,e) =$$

$$\frac{1}{R_B^j} \frac{b!}{(b-j)!} \frac{(e-i)!}{(e-i-j)!} \frac{1}{j!} P(i,0|a,b,e)$$

Substituting (115) into (116), we get

$$(117) \quad P(i,j|a,b,e) =$$

$$\frac{1}{R_A^i} \frac{1}{R_B^j} \binom{a}{i} \binom{b}{j} \binom{e}{i+j} (i!) (j!) P(0,0|a,b,e)$$

In equation (117)

$$(118) \quad \binom{a}{i} = \frac{a!}{(a-i)! i!} \quad , \quad \binom{b}{j} = \frac{b!}{(b-j)! j!}$$

$$(119) \quad \binom{e}{i, j} = \frac{e!}{(e-(i+j))! i! j!}$$

and the expression

$$(120) \quad \binom{a}{i} \binom{b}{j} \binom{e}{i, j} i! j!$$

is the number of different ways of forming  $i$  molecules of type EA and  $j$  molecules of type EB from  $a$  molecules of A,  $b$  molecules of B and  $e$  molecules of E. This is because

(121)  $\binom{a}{i}$  = number of ways of choosing  $i$  molecules of type A from  $a$  molecules of type A

(122)  $\binom{b}{j}$  = number of ways of choosing  $j$  molecules of type B from  $b$  molecules of type B

(123)  $\binom{e}{i, j} =$  number of ways of choosing  $i$  molecules of type E to pair with molecules of type A, and  $j$  molecules of type E to pair with molecules of type B, given that we have  $e$  molecules of type E.

After having made all of the above choices, we have:

- $i$  molecules of type A and  $i$  molecules of type E that can be used to form EA.
- $j$  molecules of type B and  $j$  molecules of type E that can be used to form EB.

There are then  $i!$  ways of making the pairs EA, and  $j!$  ways of making the pairs EB.

This completes the explanation of the combinatorial expression (120).

It follows from (117) that

$$(124) \quad P(i,j|a,b,e)$$

$$= \frac{\frac{1}{R_A^i} \frac{1}{R_B^j} \binom{a}{i} \binom{b}{j} \binom{e}{i,j} i! j!}{\sum_{i'=0}^a \sum_{j'=0}^b \frac{1}{R_A^{i'}} \frac{1}{R_B^{j'}} \binom{a}{i'} \binom{b}{j'} \binom{e}{i',j'} (i')! (j')!}$$

It is understood in this equation that

$$(125) \quad \binom{e}{i,j} = 0 \quad \text{if } e < (i+j)$$

In principle, then, we could use the formula (124) to replace the part of the function rapid-equilibrium that evaluates  $P$ . This would seem to be an expensive way to proceed, however, since (124) evaluates  $P$  for each pair  $i,j$  separately, without taking advantage of the simple relationships (113) & (114).

Our microscopic rapid equilibrium has a macroscopic limit, which can be studied by starting from some small volume  $V_0$  with integer values  $(n_{A^*}^0, n_{B^*}^0, n_{E^*}^0)$  of  $(n_{A^*}, n_{B^*}, n_{E^*})$ , and then scaling up to some volume  $V$  that is an integer multiple of  $V_0$ , while holding  $[A^*]$ ,  $[B^*]$ , and  $[E^*]$  constant. This gives

$$(126) \quad n_{A^*} = \frac{V}{V_0} n_{A^*}^0, \quad n_{B^*} = \frac{V}{V_0} n_{B^*}^0, \quad n_{E^*} = \frac{V}{V_0} n_{E^*}^0$$

Since  $V/V_0$  is an integer,  $n_{A^*}$ ,  $n_{B^*}$ , and  $n_{E^*}$  are integers, and moreover

$$(127) \quad [A^*] = \frac{n_{A^*}}{V} = \frac{n_{A^*}^0}{V_0}$$

$$(128) \quad [B^*] = \frac{n_{B^*}}{V} = \frac{n_{B^*}^0}{V_0}$$

$$(129) \quad [E^*] = \frac{n_{E^*}}{V} = \frac{n_{E^*}^0}{V_0}$$

so  $[A^*]$ ,  $[B^*]$ , and  $[E^*]$  are indeed constant as we scale up  $V$ .

For any particular  $V$ , we may define

$$(130) \quad [A] = \frac{\overline{n}_A}{V}, \quad [B] = \frac{\overline{n}_B}{V}$$

For large  $V$ , we expect that  $([A], [B])$  will approximately solve equations (46-47), that the approximation will improve as  $V$  increases, and that the limiting values of  $[A]$ ,  $[B]$  as  $V \rightarrow \infty$  will actually solve (46-47).

In carrying out the above procedure of scaling up  $V$ , it is important to recall that  $\overline{n}_A$  and  $\overline{n}_B$  are volume-dependent, see (105).

It would be interesting to demonstrate computationally that  $[A]$  and  $[B]$  behave in the manner described above as  $V$  increases, and perhaps even to prove analytically that  $([A], [B])$  approaches the solution to (46-47) as  $V \rightarrow \infty$ .

Remark: In (117) and in the subsequent discussion, the expression

$$(131) \quad \binom{e}{i,j} (i!) (j!)$$

could have been written as

$$(132) \quad \binom{e}{i+j} (i+j)!$$

Since both of these are equal to

$$(133) \quad \frac{e!}{(e - (i+j))!}$$

If we use (132), the combinatorial interpretation changes slightly, since we are then choosing  $i+j$  molecules of E to pair up with the  $i$  molecules of A and the  $j$  molecules of B without specifying which molecules of E will pair with A and which will pair with B. There are then  $(i+j)!$  possible pairings.

## Design of an enzyme to favor a particular direction of a reversible chemical reaction

As emphasized above, an enzyme cannot change the equilibrium constant of a reaction. Thus, it might seem that an enzyme that accelerates one direction of a reaction must accelerate the opposite direction to the same extent. This argument is incorrect!

To see this recall equation (62), which gives the net rate at which A is converted to B by the enzyme E per molecule of E, as a function of the free concentrations of A and B:

$$(134) \quad v([A], [B]) = \frac{1}{K_E} \frac{\alpha[A] - \beta[B]}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B}}$$

In this equation, the rate constants  $\alpha, \beta$  are those of the non-enzymatic reaction. They cannot be changed

by the design of the enzyme. The enzyme is characterized by the three constants  $K_A$ ,  $K_B$ ,  $K_E$ , which have units of concentration.

The rate constants  $\alpha_E$ ,  $\beta_E$  for the reactions  $EA \rightarrow EB$  and  $EB \rightarrow EA$  do not appear in the equation for  $v$ , since they have been eliminated by making use of the principle of detailed balance. They are given, however, by:

$$(135) \quad \alpha_E = \frac{\alpha K_A}{K_E}, \quad \beta_E = \frac{\beta K_B}{K_E}$$

These equations can be thought of as the definition of  $K_E$ :

$$(136) \quad K_E = \frac{\alpha}{\alpha_E} K_A = \frac{\beta}{\beta_E} K_B$$

The two definitions agree because of the principle of detailed balance.

As mentioned previously,  $v=0$  when  $\alpha[A] = \beta[B]$ , and this is exactly the same as the condition for equilibrium in the absence of the enzyme. Far from equilibrium, however, we can make the enzyme favor one direction of reaction over the other. To see this most clearly, consider separately the limits  $[A] \rightarrow \infty$  and  $[B] \rightarrow \infty$ . We get from (134)

$$(138) \quad \lim_{[A] \rightarrow \infty} v([A], [B]) = \frac{\alpha K_A}{K_E} = \alpha_E$$

$$(139) \quad \lim_{[B] \rightarrow \infty} v([A], [B]) = -\frac{\beta K_B}{K_E} = -\beta_E$$

The ratio of these limiting rates is

$$(140) \quad \frac{v(\infty, [B])}{-v([A], \infty)} = \frac{\alpha K_A}{\beta K_B}$$

We can make this ratio be whatever we like, regardless of  $\alpha/\beta$  by the proper choice of  $K_A$  and  $K_B$ !

If

$$\frac{[A]}{K_A} + \frac{[B]}{K_B} \gg 1$$

then each enzyme molecule spends most of its time bound to A or B, that is, in the form EA or EB. In that situation, equation (134) for  $\gamma$  simplifies as follows:

$$\begin{aligned}\gamma &= \frac{\alpha[A] - \beta[B]}{\frac{k_E}{K_A}[A] + \frac{k_E}{K_B}[B]} = \frac{\alpha[A] - \beta[B]}{\frac{\alpha[A]}{\alpha_E} + \frac{\beta[B]}{\beta_E}} \\ &= \sqrt{\alpha_E \beta_E} \frac{\left(\frac{\alpha[A]}{\beta[B]}\right)^{1/2} - \left(\frac{\beta[B]}{\alpha[A]}\right)^{1/2}}{\left(\frac{\beta_E}{\alpha_E}\right)^{1/2} \left(\frac{\alpha[A]}{\beta[B]}\right)^{1/2} + \left(\frac{\alpha_E}{\beta_E}\right)^{1/2} \left(\frac{\beta[B]}{\alpha[A]}\right)^{1/2}}\end{aligned}$$

Now let

$$\theta = \log\left(\frac{\alpha[A]}{\beta[B]}\right), \quad \phi = \log\left(\frac{\alpha_E}{\beta_E}\right)$$

Then

$$V = \sqrt{\alpha_E \beta_E} \frac{e^{\frac{1}{2}\theta} - e^{-\frac{1}{2}\theta}}{e^{\frac{1}{2}(\theta-\phi)} + e^{-\frac{1}{2}(\theta-\phi)}}$$

$$= \sqrt{\alpha_E \beta_E} \frac{\sinh \frac{1}{2}\theta}{\cosh \frac{1}{2}(\theta-\phi)}$$

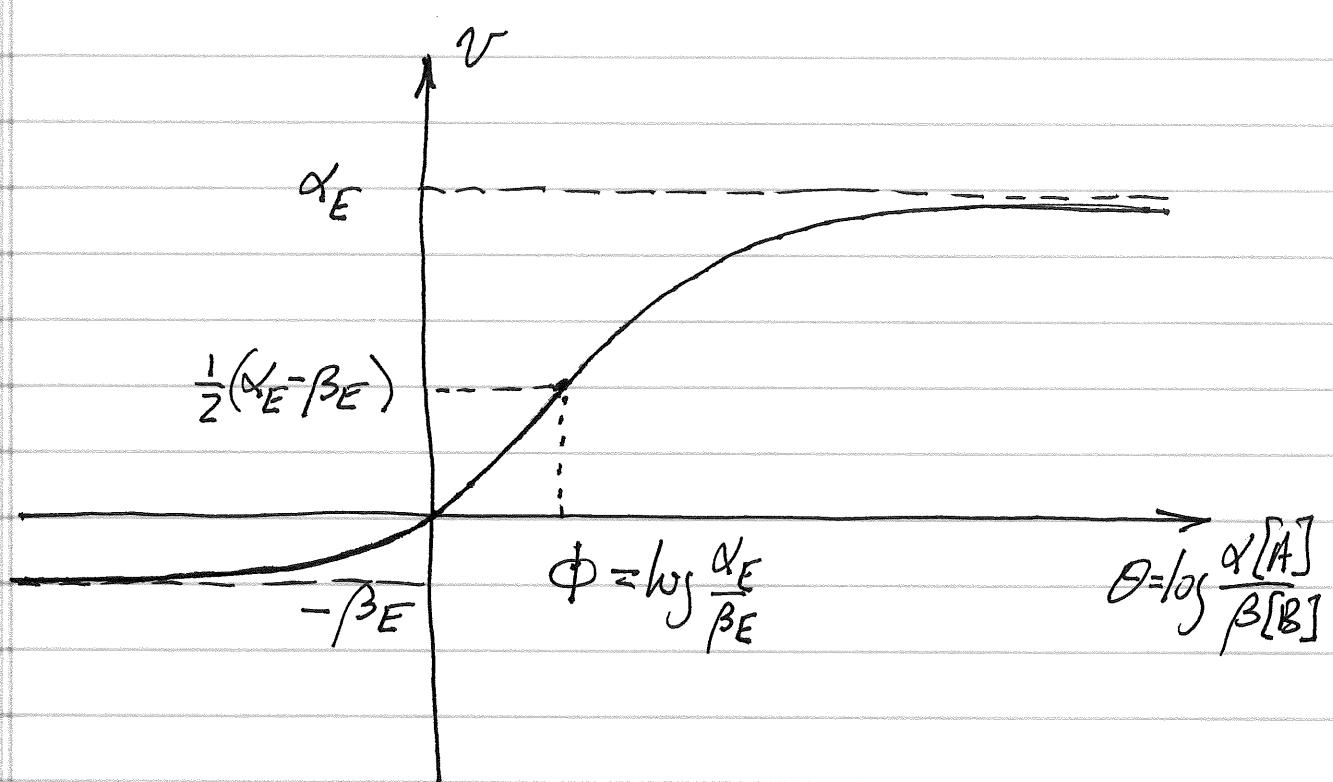
$$= \sqrt{\alpha_E \beta_E} \frac{\sinh \left( \frac{1}{2}(\theta-\phi) + \frac{1}{2}\phi \right)}{\cosh \left( \frac{1}{2}(\theta-\phi) \right)}$$

$$= \sqrt{\alpha_E \beta_E} \left( \frac{\sinh \left( \frac{1}{2}(\theta-\phi) \right) \cosh \left( \frac{1}{2}\phi \right) + \cosh \left( \frac{1}{2}(\theta-\phi) \right) \sinh \left( \frac{1}{2}\phi \right)}{\cosh \left( \frac{1}{2}(\theta-\phi) \right)} \right)$$

$$= \sqrt{\alpha_E \beta_E} \left( \sinh \left( \frac{1}{2}\phi \right) + \cosh \left( \frac{1}{2}\phi \right) \tanh \frac{1}{2}(\theta-\phi) \right)$$

$$= \sqrt{\alpha_E \beta_E} \cosh \left( \frac{1}{2}\phi \right) \left( \tanh \left( \frac{1}{2}\phi \right) + \tanh \left( \frac{1}{2}(\theta-\phi) \right) \right)$$

This gives the following plot :



which goes through the origin but has odd symmetry about the point

$$\left( \log\left(\frac{\alpha_E}{\beta_E}\right), \frac{1}{2}(\alpha_E - \beta_E) \right)$$