Kinetics of enzyme-catalysed de-symmetrisation of prochiral substrates: product enantiomeric excess is not always constant

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Abstract:

The kinetics of enzymatic de-symmetrisation were analysed for the most common kinetic mechanisms: ternary complex ordered (prochiral ketone reduction); ping-pong second (ketone amination, diol esterification, de-symmetrisation in the second half reaction); ping-pong first (diol ester hydrolysis) and ping-pong both (prochiral diacids). For plausible values of enzyme kinetic parameters, the product enantiomeric excess (ee) can decline substantially as the reaction proceeds to high conversion. For example, an ee of 0.95 at the start of the reaction can decline to less than 0.5 at 95% of equilibrium conversion, but for different enzyme properties will remain almost unchanged. For most mechanisms a single function of multiple enzyme rate constants (which can be termed ee decline parameter, *eeDP*) accounts for the major effect on the tendency for ee to decline. For some mechanisms, concentrations or ratios of starting materials have important influence on the fall in ee. For the application of enzymatic de-symmetrisation it is important to study if and how product ee declines at high conversion.

Keywords: enantiomeric excess; enzyme; kinetic mechanisms; kinetic parameters; prochiral.

INTRODUCTION

There is great interest in using enzymatic catalysis in the synthesis of homochiral molecules. An early approach was to use enantioselective enzymes in the resolution of a racemate. As such resolution reactions proceed, there are progressive changes in enantiomeric excesses (ee), that of the product falling while that of residual starting material increases. In a classic study of the kinetics of resolution, Chen & Sih [1] showed how these changes reflected an unchanging characteristic of the enzyme, the enantioselectivity E, equal to the ratio of k_{cat}/K_m values for the two enantiomers. The Chen & Sih equations are widely used to analyse the progress of resolution reactions. Chen & Sih also described the influence of the equilibrium constant where the enzymatic reaction was not completely irreversible [2].

A limitation of enzymatic resolution is that the maximum yield of the desired enantiomer is the 50% contained in the starting racemate. This is one reason for the current greater interest in enzymatic de-symmetrisation reactions, in which a pro-chiral substrate is used [3-7]. The reaction generates a new chiral centre, and the enzyme shows enantiospecifity in making predominantly one enantiomer as product. In this case yields of the favoured enantiomer can approach 100%. Some popular reactions are reduction of prochiral ketones to chiral secondary alcohols, transamination of prochiral ketones to chiral amines, hydrolysis of symmetrical diesters to a chiral monoester, and esterification of prochiral diacids or diols.

In de-symmetrisation reactions, the enzyme initially produces the two product enantiomers in an unchanging ratio (with one usually strongly favoured). Hence it is usual to quote the enantiomeric excess (ee) of the product as if it were a characteristic property of the enzyme under given conditions, remaining constant up to high conversions. A search for all papers over the last year or so dealing with enzymatic de-symmetrisation of prochiral compounds showed that all quote a value of ee to characterise the behaviour for any enzyme and reaction conditions [8-31]. Sometimes this

comes with a conversion or yield at which it was obtained. However, I have heard anecdotally from people performing such reactions that the ee can appear to decline as the reaction proceeds. (I have been unable to find any published report of this effect.)

Perhaps because of the view that the kinetics of such reactions are straightforward, they have received little study. Smith et al [32] and more recently Yamane [33] have studied the kinetics of example reactions, but without progress data for ee. Kroutil et al [34] derived a model for consecutive reactions, but the de-symmetrisation reaction was assumed to produce enantiomers in a fixed ratio throughout. The current paper presents a study of the kinetics of enzymatic de-symmetrisation reactions that explicitly examines the progress of ee. It shows that for plausible enzyme parameters, the product ee can decline at high conversion, often quite substantially. Understanding this effect, and features of the enzyme and reaction conditions that can minimise it, is important for the optimal design of enzymatic de-symmetrisation reactions.

RESULTS & DISCUSSION

Scheme 1 shows the kinetic mechanisms that cover most applications of enzymatic de-symmetrisation. These show that the mechanisms necessarily include a pathway of elementary reactions catalysed by the enzyme that brings about racemisation of the product. This will always be possible unless either the reaction is completely irreversible, or the reactions leading to the less favoured product are completely absent. In most of the applications the overall reaction is significantly reversible, with an equilibrium constant that is not enormously larger than 1. And in many cases there is noticeable formation of the less favoured enantiomer, with product ee values of 0.98 or less. Even an ee of > 0.99, as often reported, may not be enough to make formation of the less favoured enantiomer always negligible. It should be clear that formation of product with a fixed ee is an initial rate phenomenon, which may not persist to high conversion. Eventually the ee must

3

fall towards the ultimate equilibrium value of 0, although the timescale for this depends on details of

the enzyme kinetics.

Ordered, second

Ping-pong, second

Ping-pong, first

Ping-pong, both
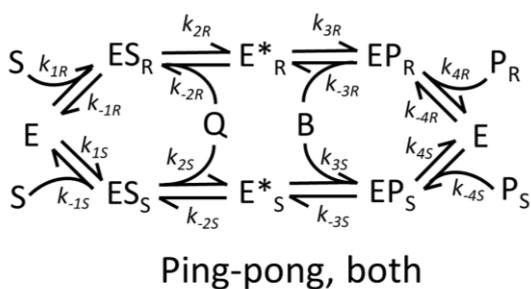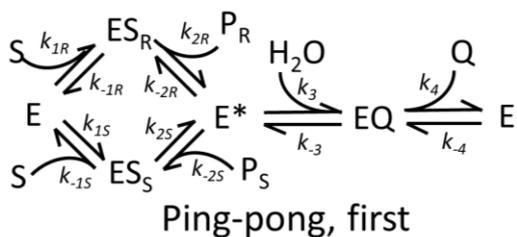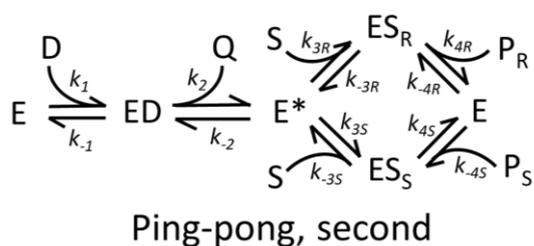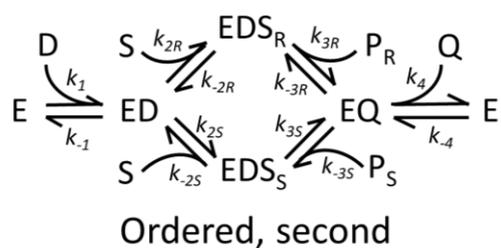
Scheme 1. Kinetic mechanisms. In each case E represent the free enzyme, other species starting E

are other enzyme forms, S is the prochiral substrate, D, B or H_2O is the second substrate, Q is the

side-product, and P_R and P_S are the two enantiomeric chiral products. Elementary kinetic rate

constants are shown next to each reaction arrow.

To understand the range of possible behaviour, progress curves for conversion and ee were calculated for a wide variety of possible enzyme kinetic parameters. The calculation used integration of the fundamental differential equations that describe the relevant kinetic mechanism. For some plausible kinetic parameters, the results show a substantial decline in product ee as reactions are run to high conversion. Fig. 1 below shows some examples. It is clear that in such cases a single value of product ee does not characterise the enzyme and reaction behaviour. An ee value observed under some particular circumstances may be a poor guide to performance under others. In particular, a good ee found early in the reaction may be a poor guide to a preparative reaction where high conversion is desirable.

The details of behaviour depend on the kinetic mechanism followed by the enzyme and reaction of interest. Most of the reported examples will follow one of the 4 kinetic mechanisms shown in Scheme 1. The behaviour is somewhat different in each case, so these kinetic mechanisms need to be considered separately. For reference in this paper, the kinetic mechanisms are labelled using two words, referring to the overall type and the location of the enantiospecific step(s).

- "Ordered, second". This is the ternary complex ordered mechanism followed by most dehydrogenases and keto-reductases. The reductant (usually NADH or NADPH) has to bind first to the enzyme, followed by the prochiral ketone in the second step. The chiral products are then released before the oxidised co-product.

- "Ping-pong, second". Followed by most transaminases and lipase or esterase catalysed acylation of prochiral diols. The enzyme reacts with an amino or acyl donor, releasing a first co-product, to give an aminated or acylated enzyme intermediate. This then reacts with the prochiral ketone or diol to generate the chiral products.

- "Ping-pong, first". Followed in most lipase or esterase hydrolyses of prochiral diol esters. The enzyme reacts enantiospecifically with the ester to release a chiral product, leaving the acyl group attached to the active site. In a second stage the achiral acyl group undergoes hydrolysis by water. In de-symmetrisation of diols (and diacids, below) kinetic amplification can sometimes be exploited to raise product ee [7]. This involves selective further reaction of the unwanted product enantiomer. Including kinetic amplification in the model would involve doubling the number of enzyme rate constants, which was considered excessive complication at this stage.

- "Ping-pong, both". Followed in most lipase or esterase reactions of prochiral diacids: either hydrolysis of their esters, or esterification of the free acids. The enzyme reacts enantiospecifically with the ester or acid. But now the acyl enzyme can be in either stereoisomeric form, so the kinetics are different for all subsequent steps.

It might be hoped that behaviour in each of these cases could be predicted intuitively based on the extensive published analysis of the kinetics of the ternary complex ordered and Ping-Pong mechanisms. However, I was unable to do so. The effects reported were mainly identified by empirical observation of the results of simulating enzyme reaction progress, varying input parameters over likely ranges. Some effects could be rationalised after they had been identified.

The next section explains the selection of parameters that describe enzyme properties important in determining the time course of product ee. Readers who are mainly interested in seeing the possible behaviour can skip this section, perhaps coming back later to see the derivation of enzyme parameters.

Enzyme parameters affecting behaviour

The overall kinetic behaviour is governed by at least 12 elementary rate constants that appear in the appropriate kinetic mechanism. The reaction progress was determined by integrating the differential

equations incorporating these rate constants. The value of each of these rate constants depends on the enzyme and reaction conditions chosen for use.

However, it is more informative to consider the behaviour as a function of groups that each combine several elementary rate constants, as normal in enzyme kinetics. Firstly, there are constraints that mean the enzyme cannot vary all these rate constants independently. A Haldane relationship makes a combination of rate constants equal to the equilibrium constant of the overall reaction, which the enzyme is of course unable to change. A different combination of rate constants must equal the equilibrium constant for the isomerisation of one product enantiomer into the other, which is necessarily 1 (in an achiral medium).

It would thus be possible to treat all but 2 of the rate constants as independent input parameters. However, this is not the most sensible approach. Instead, combinations of rate constants can be related to values more likely to be known by users of relevant enzymes. And other combinations prove to have a dominant influence on the type of behaviour observed, so that this can be largely captured using fewer input parameters.

Almost always the ratio of product enantiomers formed in the initial stages of reaction will be known from measurements, so this is a sensible input parameter, equal to a combination of elementary rate constants. Often estimates will be available for the K_M values of the enzyme under initial rate conditions in the forward direction. These K_M values can also be used as input parameters, again equal to a combination of rate constants. Finally, multiplying all elementary rate constants by the same factor will not change the shape of the progress of product formation and enantiomeric excess, just the timescale over which this occurs. Hence one elementary rate constant may be set to an arbitrary value, with others effectively expressed as a multiplier times this value. This can be seen as setting the timescale for the reaction.

This leaves at least 6 more independent parameters that determine the enzyme behaviour. The grouping of elementary rate constants to define these parameters is a matter for judgment. The ones selected in this study were based in part on mathematical analysis of the rate equations, using the usual quasi-steady state approximation. This showed that certain groupings were common in the derived equations for the progress of product formation. Parameter choices were also influenced by empirical observation of effects on the decline in product ee as the reaction progressed. It proved possible to choose sets of parameters such that most of the influence came from the values of just a few parameters, with others having little effect. Hence most of the behaviour could be understood in terms of just a few parameters, giving a simpler overall picture. A further criterion was a preference for dimensionless parameters, like the ratio of two elementary rate constants (both first order or both second order). Dimensionless parameters make it easier to represent all possible behaviour of diverse enzymes. Finally, many parameters could be defined in such a way that there were expected to be usually of the order of magnitude of 1, so assigning values for kinetic simulations was easier.

One type of grouping of rate constants was found to come up regularly in the analytical solution of equations resulting from the quasi-steady state approximation. These had the form of a k_{cat}/K_M ratio (a specificity constant), but just for a part of the overall reaction. For example, in a Ping-Pong reaction, if we think of the first two steps independently, the K_M would be $(k_{-1} + k_2)/k_1$, and the k_{cat} would be k_2, so the specificity constant equivalent is $k_1 \cdot k_2/(k_{-1} + k_2)$. This is the sort of grouping that came out from the equations. It is important to note, however, that these are not equal to the true k_{cat} and K_M of the enzyme that might be measured by study of the overall reaction. Hence they are referred to as pseudo-specificity constants. They are labelled by "SC", usually followed by R or S (for the two enantiomers), then f or b to indicate the forward or backwards progress through the relevant two steps. SC values were normally used for the two steps that involve enantioselectivity (2 and 3 for the Ordered, second mechanism, 3 and 4 for Ping-Pong, second, 1 and 2 for Ping-Pong,

first.) For the Ping-Pong, both mechanism, there are two sets of SC values, *SCRf* etc for steps 1 and 2, and *SCR2f* etc for steps 3 and 4.

These pseudo-specificity constants have units of $M^{-1} s^{-1}$, so were not used directly as input parameters to simulations. However, their ratios were used, sometimes in combination with other parameters. So for example the ratio *SCRf/SCSf* was found to give (in most cases) the ratio of initial rates of formation of the two enantiomer products. Hence it can be named as an *E* value for the enzyme, by analogy with the enantioselectivity in resolution reactions. Similarly the ratio *SCRf/SCRb* was found to have an important influence in all cases – it is given the symbol *SCRf/b*. For the Ping-Pong, both mechanism, the ratio *SCR2f/SCS2f* is also relevant, and was given the symbol *E2*.

Behaviour for "ordered, second" kinetics

This is the kinetic model expected for most dehydrogenases and ketoreductases carrying out reduction of prochiral ketones. In such reactions it is usual to recycle the co-substrate, usually NADH or NADPH. To give an idea of behaviour under co-substrate (cofactor) recycling, reaction progress was simulated with concentrations of D (NAD(P)H) and Q (NAD(P)$^+$) constant throughout the reaction. This is a fair approximation for what happens with efficient recycling. Fig. 1 shows the simulated progress of a reaction for a set of plausible conditions and enzyme properties. In all cases the product ee falls noticeably from the initial value of 0.95 as the reaction progresses. This decline is substantially faster for lower values of the ratio of D to Q. As this ratio decreases, the progress of conversion also slows more, as expected, as the reaction approaches a lower equilibrium conversion. The enzyme parameter values chosen for Fig. 1 are ones for which ee does decline significantly; the range of possible behaviour is presented below. The input parameter values are defined for the case where the R enantiomer is favoured. But the behaviour is actually completely symmetric, and simply exchanging all R for S and vice versa covers cases where S is favoured.
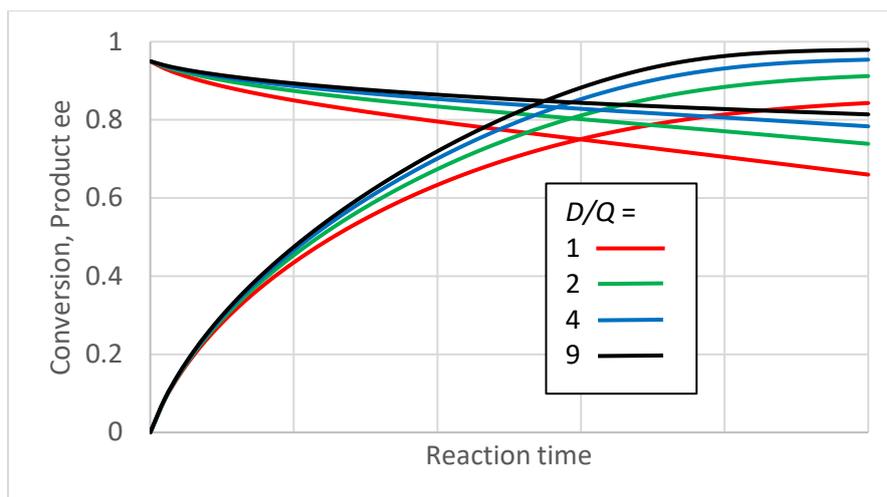
Fig. 1. Reaction progress for "ordered, second" kinetics and the effect of D/Q (e.g. NADH/NAD+)

ratio. S_0 = 0.2 M, Total $D + Q$ = 0.01 M, K_{eq} = 5, E = 39, K_{MD} = 0.001 M, K_{MS} = 0.001 M, k_4 = 1000 s^{-1},

$SCRf/b$ = 1, k_{3R}/k_4 = 1, $k_{3R}/(E \cdot k_{3S})$ = 1, k_{-4}/k_1 = 1, k_{-2R}/k_{-1} = 1, $E \cdot k_{-2S}/k_{-1}$ = 1. For the calculation the

enzyme concentration was adjusted such that all initial rates were the same. Hence the reaction

time is essentially dimensionless, and no values are given on the axis. The K_{eq} value here is found for

reduction of simple ketones by NADH at pH around 7.5 [35].

To aid comparison of reactions under different conditions and enzyme parameters, we can examine

the product ee at a conversion that is 95% of that expected at equilibrium (for a fully enantiospecific

reaction). This is a typical conversion that might be selected for a preparative reaction. For the

reactions in Fig. 1, these ee values are 0.725, 0.782, 0.816 and 0.836 as D/Q is increased from 1 to 9.

All of these ee values are of course substantially lower than 0.950 found at the beginning of the

reaction. Under cofactor recycling, the ratio D/Q can be controlled by the excess of ultimate

reductant chosen, and also by its identity. Changing the equilibrium constant of the reaction will also

affect the equilibrium conversion, but has a much smaller effect on the product ee. For D/Q = 2,

doubling the K_{eq} from 5 to 10 only increases ee at 95% of equilibrium conversion from 0.782 to

0.793. Changing the total concentration of D + Q had negligible effect, provided the ratio remained the same.

The example of Fig. 1 was for a case where the initial product ee was 0.95 (E = 39), because it is easier to see changes in ee on the graph. Fig. 2 shows what happens for other E values, and also the effect of the initial concentration of the prochiral starting material, which was found to have an important effect. As can be seen there are noticeable falls in product ee by the time the reaction approaches equilibrium, for all E values. At a starting material concentration of 1 M, product ee falls from the values early in the reaction of 0.99 (E = 199) and 0.98 (E = 99) to 0.948 and 0.834 respectively. For fixed enzyme properties, the product ee near equilibrium also falls noticeably with increase in prochiral starting material concentration. It would be quite common for a reaction found to be useful at low concentration to be repeated at higher concentration for preparative purposes. As this graph shows there might be an unwelcome and unexpected fall in product ee obtained. To study the effects of other enzyme parameters, simulations were routinely run for an E value of 39 (initial product ee 0.95), which makes changes clearer to display.
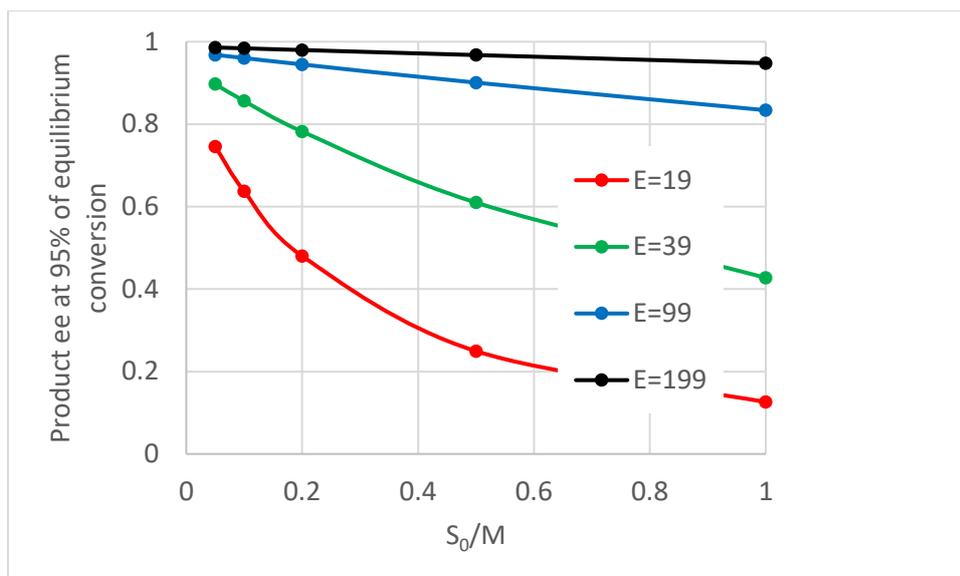
Fig. 2. Effect of initial starting material concentration and enzyme E value for "ordered, second" kinetics. $D = 0.00333$ M, $Q = 0.00167$ M, $K_{eq} = 5$, $E = 39$, $K_{MD} = 0.001$ M, $K_{MS} = 0.001$ M, $k_4 = 1000$ s^{-1}, $SCRf/b = 1$, $k_{3R}/k_4 = 1$, $k_{3R}/(E \cdot k_{3S}) = 1$, $k_{-4}/k_1 = 1$, $k_{-2R}/k_{-1} = 1$, $E \cdot k_{-2S}/k_{-1} = 1$.

A number of other enzyme parameters were found to have negligible effects on product ee at 95% equilibrium conversion: K_{MD}, k_{-2R}/k_{-1}, $E \cdot k_{-2S}/k_{-1}$. As expected, k_4 has absolutely no effect, simply making all changes happen faster or slower – so changes in k_4 are compensated by changes in simulated enzyme concentration, in order to keep the normalised fixed initial rate.

The effects of the remaining enzyme parameters could be largely summarised by two groupings, the product $K_{MS} \cdot SCRf/b$ and the ratio $E \cdot k_{3S}/k_4$ (actually the ratio of input parameters k_{3R}/k_4 and $k_{3R}/(E \cdot k_{3S})$). This behaviour is shown in Fig. 3. As can be seen, if $K_{MS} \cdot SCRf/b$ is sufficiently large, and $E \cdot k_{3S}/k_4$ is sufficiently small, the product ee will remain high throughout the reaction, remaining around 0.94 even at 95% of the equilibrium conversion, only slightly less than its initial value of 0.95. However, if the enzyme does not have these properties, the product ee can drop substantially as the reaction progresses, even to values that are of little preparative value. In fact, most of the effect of these two

parameters is captured by their ratio, so the group $E \cdot k_{3S}/k_4$ divided by $K_{MS} \cdot SCRf/b$ acts a single parameter that describes the tendency for ee to decline with a particular enzyme, which can be referred to as "ee decline parameter (*eeDP*)". If the value of *eeDP* for a particular enzyme is small, the decline in ee at high conversion will be small and perhaps negligible. However, for *eeDP* greater than 100 M^{-1}, and particularly greater than 1000 M^{-1}, the product ee at preparative conversion can be substantially lower than expected from the *E* value. Hence it would be valuable to estimate *eeDP* for candidate enzymes. Measurements of product ee at 2 or 3 different conversions may be sufficient to give a rough estimate, which would be valuable for planning a synthesis. When selecting the best enzyme, *eeDP* may be as important to know as the E value. Note that *eeDP* values greater than 100 M^{-1} are probably quite common, due to a K_{MS} of less than about 0.01 M. It would also be possible to give *eeDP* units based on mM, so that the limiting values become 0.1 and 1 mM^{-1}. Clearly *eeDP* is likely to be large where K_{MS} is small. Intuitively a low K_{MS} for the prochiral starting material might be thought a good thing, and it may well be in terms of reaction rate. But this analysis shows that it is undesirable in terms of maintaining good product ee through the reaction progress. One other enzyme parameter, k_{-4}/k_1, had a smaller but noticeable effect on the decline in product ee, with higher values making this worse (greater).
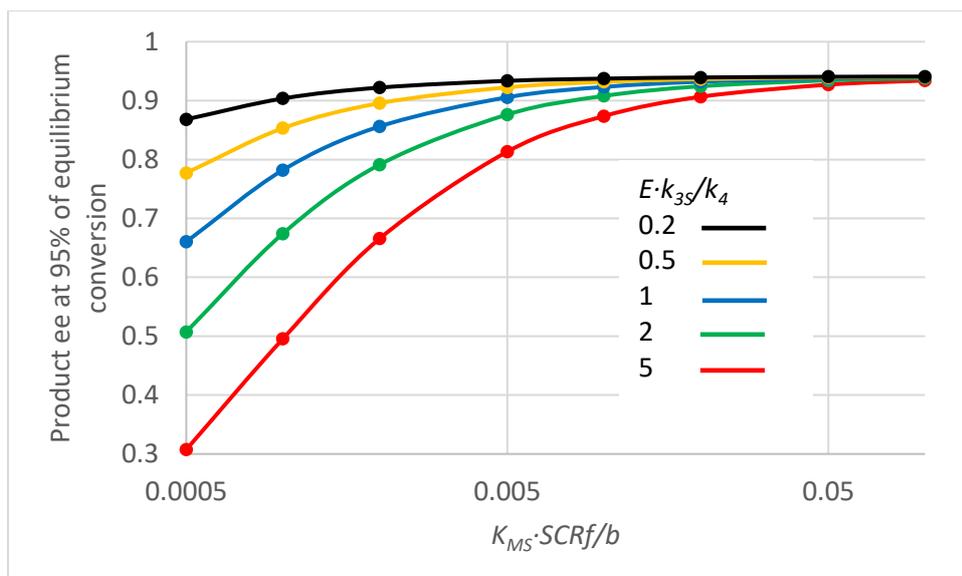
Fig 3. Effects of key enzyme parameters on fall in product ee during reaction for "ordered, second" kinetics. S_0 = 0.2 M, D = 0.00333 M, Q = 0.00167 M, K_{eq} = 5, E = 39, K_{MD} = 0.001 M, K_{MS} = 0.001 M, k_4 = 1000 s^{-1}, k_{3R}/k_4 = 1, k_{-4}/k_1 = 1, k_{-2R}/k_{-1} = 1, $E \cdot k_{-2S}/k_{-1}$ = 1. For this plot the groups shown were varied by changing $SCRf/b$ and $k_{3R}/(E \cdot k_{3S})$. However, the values of ee at 95% of equilibrium conversion were almost identical if the values of the same groups were varied by changing K_{MS} and k_{3R}/k_4.

When the simulation parameters incorporated in *eeDP* are expanded in terms of elementary rate constants, some cancellations occur:-

$$eeDP = \frac{\dfrac{E.k_{3S}}{k_4}}{K_{MS}.SCRf/b} = \frac{\dfrac{E.k_{3S}}{k_4}}{K_{MS}.\dfrac{k_{2R}.k_{3R}}{k_{-2R}.k_{-3R}}} = \frac{E.k_{3S}.k_{-2R}.k_{-3R}}{K_{MS}.k_{2R}.k_{3R}.k_4}$$

It is possible also to express E and K_{MS} in terms of elementary rate constants and substitute in this expression, but the result does not offer any clear interpretation (see Supporting Information). It makes intuitive sense that higher values of backwards rate constants for the R enantiomer will increase the tendency for ee to decline as the reaction approaches equilibrium. The ratio $k_{-2R}.k_{-3R}./(k_{2R}.k_{3R})$ will tend to be greater for smaller values of K_{eq}, but the relationship is not

14

automatic, as the full expression for K_{eq} also includes $k_{-1}.k_{-4}./(k_1.k_4)$. A change in K_{eq} necessarily means a change in some elementary rate constants, but enzyme properties determine the details of these changes, subject to maintaining the overall thermodynamic relationship. With the parameter set used in these simulations, increase in K_{eq} alone mainly caused reductions in k_{-1} and k_{-2R}, partly offset by an increase in k_{-3R}.

The examples of Figs 2 and 3 above were for a case where *eeDP* was 1000 M^{-1}, so where decline in ee was relatively strong. This was chosen in order to make the effects clearly visible. It is possible that many enzymes used in practice have values of the ratio such that falling ee is not a major issue. But the values chosen here are not exceptional, and many enzymes used in practice are likely to face some problem with decline in ee at high conversion.

It is unlikely that people working with these enzymes will start with an idea of the magnitude of *eeDP* or all the parameters incorporated in it. However, *eeDP* might be estimated by accurate data for product ee at different stages of the reaction and for different starting material concentrations. That would allow predictions for what product ee might be found under different conditions. It should be clear from the behaviour shown here that quoting a single value of product ee can be very misleading about how an enzyme will perform under different conditions.

Behaviour for "ping-pong, second" kinetics.

This kinetic mechanism should apply for most lipase or esterase catalysed acylation of a prochiral diol. It will also apply to most transaminases used with a prochiral ketone as amino group recipient. Fig. 4 shows some progress curves for this mechanism with plausible values of enzyme parameters (admittedly chosen such that ee does decline, see below). As can be seen, for these parameters the product ee falls quite rapidly from its initial 0.95 as the reaction proceeds. The decline is less if there is a higher ratio of D (donor of e.g. amino or acyl groups) to S, the prochiral starting material (ketone or diol). The higher ratio also increases the equilibrium conversion, of course, and shortens the time

to reach it. Hence the product ee values at 95% of the equilibrium conversion are 0.687, 0.768, 0.840 and 0.890 for D_0/S_0 of 1, 2, 4 and 8 respectively. These values were unaffected by the actual concentration S_0, provided D_0/S_0 was kept the same.
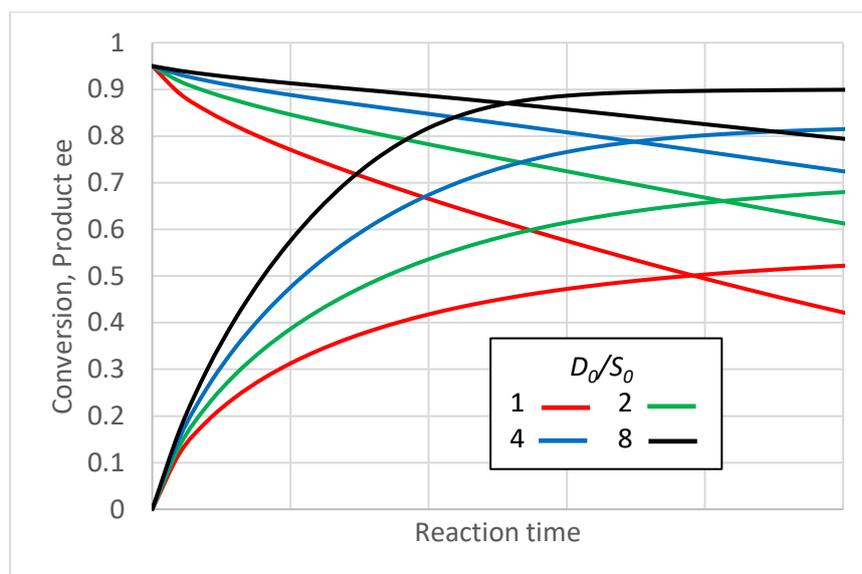

Fig. 4. Reaction progress for "ping-pong, second" kinetics, and the effect of ratio of donor to prochiral substrate. S_0 = 0.2 M, K_{eq} = 1, E = 39, K_{MD} = 0.2 M, K_{MS} = 0.01 M, k_2 = 1000 s^{-1}, $SCRf/b$ = 1, k_2/k_{4R} = 1, $E \cdot k_{4S}/k_{4R}$ = 1, k_{-1}/k_2 = 1, k_{3R}/k_{-4R} = 1, k_{3S}/k_{-4S} = 1. Again enzyme concentration was adjusted such that all initial rates were the same. The K_{eq} will be close to 1 for many transaminase or transesterification reactions, but may be substantially higher if activated donors are used.

Now consider the effects of enzyme properties on product ee at 95% of equilibrium conversion. It turns out that almost the entire effect (for a given E value) can be accounted for by the value of a single group of input parameters, K_{MD} divided by $K_{MS} \cdot SCRf/b$. So again this can be termed an "ee decline parameter (*eeDP*)". Fig. 5 shows that for small values of *eeDP*, the product ee remains fairly close to the initial value of 0.95. But for *eeDP* greater than 1, and particularly greater than 10, the

product ee close to equilibrium declines substantially, often to values that are of little preparative

value. For fixed $eeDP$, Fig. 5 shows a much weaker effect of K_{eq}, and for high values of $eeDP$,

increasing K_{eq} actually worsens the decline in product ee (for fixed $eeDP$). At low $eeDP$, increasing K_{eq}

improves product ee close to equilibrium, getting nearer the initial value of 0.95 (0.946 for $eeDP$ =
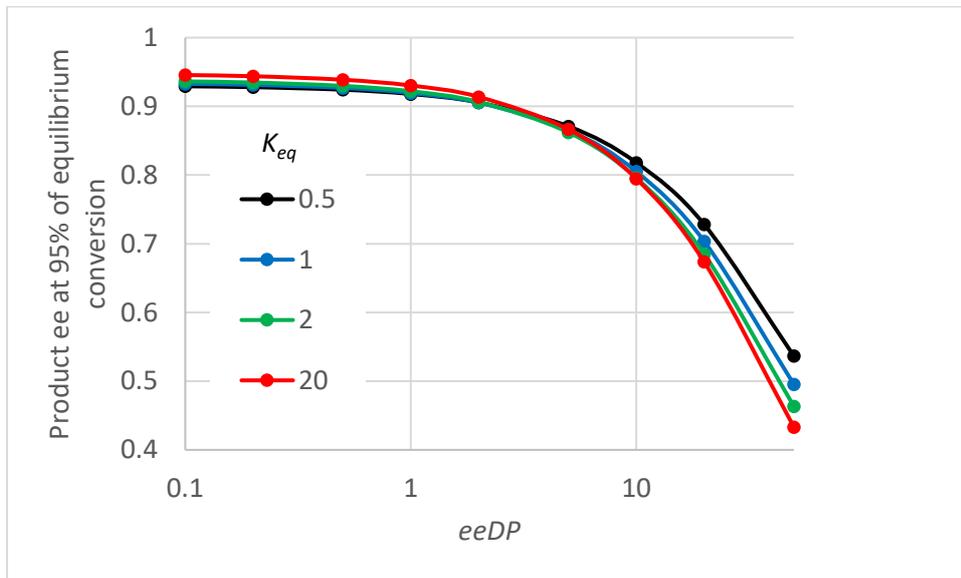
0.1 and K_{eq} = 20).

Fig 5. Effect of key ee decline parameter ($eeDP$) of enzyme on product ee for "ping-pong, second"

kinetics. S_0 = 0.2 M, D = 0.4 M, E = 39, K_{MS} = 0.01 M, k_2 = 1000 s^{-1}, SCRf/b = 1, k_2/k_{4R} = 1, $E \cdot k_{4S}/k_{4R}$ = 1,

k_{-1}/k_2 = 1, k_{3R}/k_{-4R} = 1, k_{3S}/k_{-4S} = 1. For this plot $eeDP$ was varied by changing K_{MD}. But ee at 95% of

equilibrium conversion is almost exactly the same if $eeDP$ is varied by changing K_{MS} (0.001 to 10 M)

or $SCRf/b$ (0.1 to 10).

From the analytical expression for initial rate it is found that:

$$\frac{K_{MD}}{K_{MS}} = \left(\frac{SCRf}{SCD} + \frac{SCSf}{SCD}\right) = \frac{SCRf}{SCD} \cdot \left(1 + \frac{1}{E}\right)$$

And hence we can write *eeDP* as:

$$eeDP = \frac{SCRb}{SCD}\cdot\left(1+\frac{1}{E}\right) = \frac{k_{-3R}.k_{-4R}}{(k_{4R}+k_{-3R})}\cdot\frac{(k_2+k_{-1})}{k_1.k_2}\cdot\left(1+\frac{1}{E}\right)$$

The ratio of pseudo-specificity constants here governs the competition between product PR and donor D in reacting with the free enzyme. The relative rates will be given by *SCRb·PR* divided by *SCD·D*. It makes sense that a large value of this ratio will lead to a faster decline in ee as the reaction approaches equilibrium. There will be some tendency for *eeDP* to be larger for smaller values of K_{eq}, because of backwards rate constants in the numerator and forward ones in the denominator. However, there will not be a fixed relationship, as a change in K_{eq} may actually be reflected in other rate constants. With the input parameter definitions used here, changes in K_{eq} are largely accommodated by changes in k_{-2}, and a given enzyme may adjust to changes in K_{eq} in many other ways.

A list of other enzyme parameters were found to have no noticeable effect on product ee at 95% of equilibrium conversion: k_2, k_2/k_{4R}, $E\cdot k_{4S}/k_{4R}$, k_{-1}/k_2, k_{3R}/k_{-4R}, k_{3S}/k_{-4S}.

Behaviour for "ping-pong, first" kinetics.

This model will normally apply in enzymatic hydrolysis of prochiral diol esters, so simulations were run for the case where the second substrate (H_2O in this case) is in constant excess. As can be seen from Fig 6, the prochiral substrate concentration S_0 has a major effect on whether product ee falls substantially below the initial value. For low initial substrate concentration or high K_M, the ee remains close to its initial value of 0.95. However, for plausible lower K_M values, and preparatively more attractive substrate concentrations, the product ee obtained on approach to equilibrium can be greatly reduced. In fact the value of product ee is similar for the same value of S_0/K_M, regardless of the individual values. It begins to fall noticeably for $S_0/K_M > 1$, and is greatly depressed for $S_0/K_M >$

20. Note again that a low K_M value for the prochiral substrate would normally be seen as a good characteristic of the enzyme, but in fact may lead to problems with declining ee at high conversion.
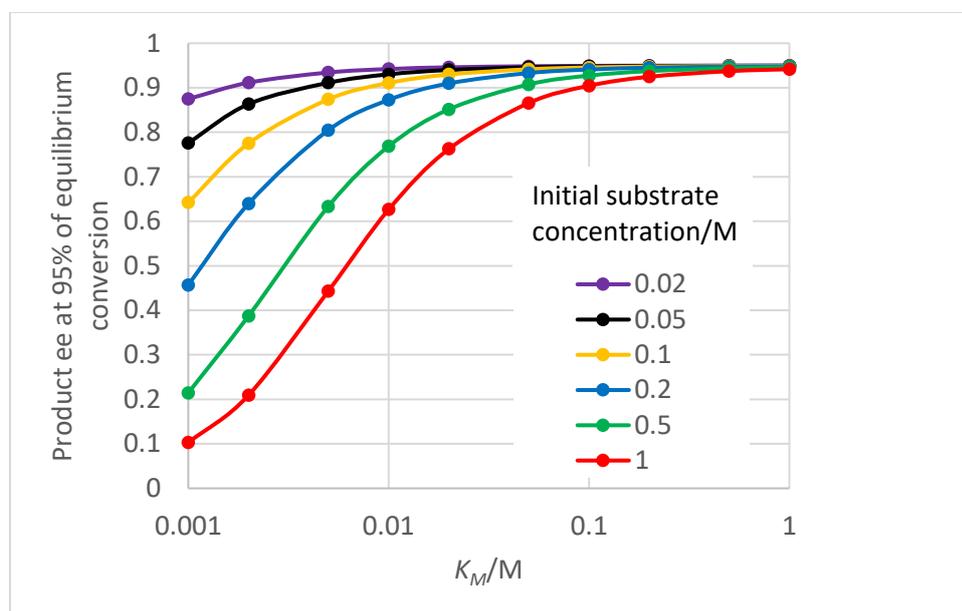

Fig 6. Effects of pro-chiral substrate concentration and its K_M value for "ping-pong, first" kinetics.

Input parameters were: K_{eq} = 15 M, E = 39, k_4 = 1000 s^{-1}, $SCRf/b$ = 5, k_{2R}/k_4 = 1, $k_{2R}/E \cdot k_{2S}$ = 1, $k_3 \cdot [H_2O]/k_4$ = 1, $k_{-4} \cdot K_{eq}/k_4$ = 1, k_{-1R}/k_{-3} = 1, $E \cdot k_{-1S}/k_{-3}$ = 1. The K_{eq} here is defined omitting the water concentration, so has units. For diol ester hydrolysis K_{eq} will probably depend significantly on the positions of the hydroxyl groups with respect to each other. A value around 40 M was found for glycerol dioctanoate [35].

Focussing on enzyme properties, it is again possible to identify a single ee decline parameter (*eeDP*) that accounts for the major effects on the fall in product ee as the reaction proceeds. As Fig. 6 would suggest, one component of this is the K_M value, but the *eeDP* here is defined as the reciprocal of K_M times *SCRf/b*. However, in this case other enzyme parameters were found to have a noticeable, although smaller, effect on the fall in ee. Fig. 7 shows the effect of *eeDP* and also the next most influential parameter $k_{-4} \cdot K_{eq}/k_4$. For *eeDP* of 1 M^{-1} or less, product ee remains close to the initial

value of 0.95 throughout. But for *eeDP* greater than 10 M^{-1} the product ee can be substantially

reduced. For a given value of *eeDP*, the reduction in product ee at high conversion is less for larger

value of $k_{-4} \cdot K_{eq}/k_4$, that is when the reaction of the enzyme with the acid by-product is more

kinetically favoured. A referee has pointed out that product ee at high conversion might be

improved by adding a nucleophile that deacylates E* better than water does. Full analysis of the

kinetics shows that the K_M value is actually given by *SCRf* multiplied by a rather complicated function

of other rate constants (see Supporting Information). Hence *eeDP* is actually equal to *SCRb* times this

function of other rate constants. It is intuitively reasonable that there should be an important role

for this pseudo-specificity constant, which applies to the reaction of the favoured enantiomer

product with the acyl enzyme to regenerate the prochiral diol starting material. Three other

parameters had small effects on the decline in product ee at high conversion: k_{2R}/k_4, $k_{2R}/E \cdot k_{2S}$ and

K_{eq}. Four others were found to have no detectable effect: k_4, $k_3 \cdot [H_2O]/k_4$, k_{-1R}/k_{-3} and $E \cdot k_{-1S}/k_{-3}$.
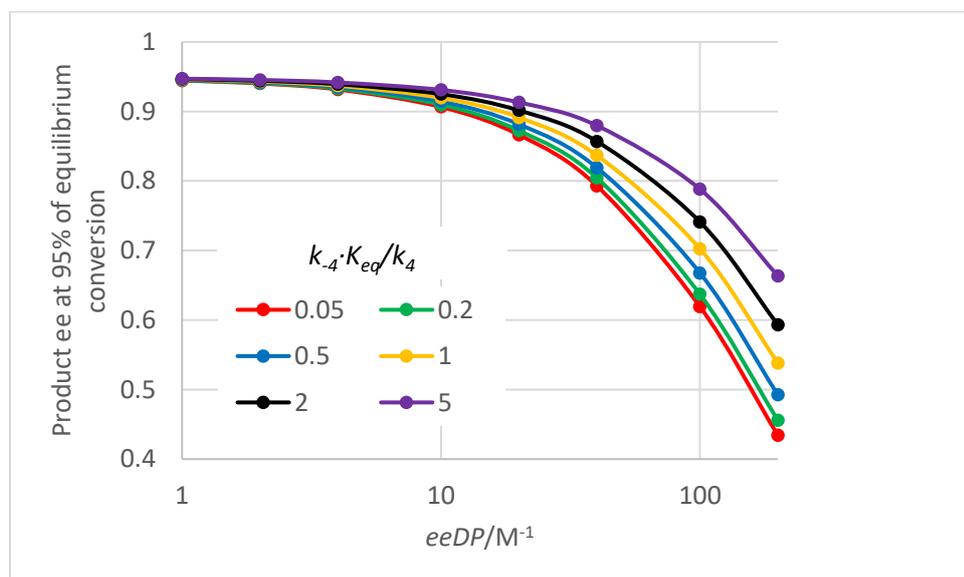

Fig. 7. Effect of *eeDP* and $k_{-4} \cdot K_{eq}/k_4$ on product ee at high conversion for "ping-pong, first" kinetics.

S_0 = 0.2 M, K_{eq} = 15 M, E = 39, k_4 = 1000 s^{-1}, *SCRf/b* = 5, k_{2R}/k_4 = 1, $k_{2R}/E \cdot k_{2S}$ = 1, $k_3 \cdot [H_2O]/k_4$ = 1, k_{-1R}/k_{-3}

= 1, $E \cdot k_{-1S}/k_{-3}$ = 1. For this plot, different values of *eeDP* (= $1/(K_M \cdot SCRf/b)$) were obtained by varying

K_M, but similar product ee values were found by varying *SCRf/b* instead.

Behaviour for "ping-pong, both" kinetics.

This mechanism will be found for reactions of prochiral diacids using most lipases and esterases: either hydrolysis of their diesters, or synthesis of a monoester from the free acid. Because this kinetic mechanism has different reactions for the two enantiomers at every stage, there are no less than 16 elementary rate constants involved. The same model applies to both hydrolysis and esterification reactions, but because the initial reactant concentrations are very different in the two cases, the behaviour is discussed separately.

In the case of diester hydrolysis, product Q is an alcohol and reactant B is water, normally present at high concentration such that it hardly changes as the reaction proceeds. For such reactions, the conclusion from the simulations is relatively simple. There seem to be no plausible enzyme parameters for which the product ee drops substantially below the value found at the start of the reaction. For an E value of 39, such that the initial product has ee of 0.95, it did not drop below 0.919 at 95% of equilibrium conversion for any combinations of parameters tested.

In the case of esterification, product Q is water, and will normally be present at a substantial initial concentration (even though a non-aqueous medium is usual). Reactant B will be an alcohol. Fig 8 shows some progress curves for plausible conditions and enzyme parameters under which product ee deviates noticeably from the 0.95 expected for the value of E. In this case even the initial product ee differs from 0.95 except when the concentration of B is high. This is surprising behaviour, but its occurrence was also supported by analytical solution of the quasi-steady state equations. These solutions suggest that the key parameter is actually the ratio of B to Q (i.e. B to H_2O). Fig. 9 shows how the initial ee depends on the ratio B_0/Q_0, and the most influential enzyme parameter, which can again be termed ee decline parameter (*eeDP*). In this case empirical analysis showed that this parameter was $E \cdot SCSf/b$ divided by ($E2 \cdot SCRf/b$). Note how for small values of *eeDP* and B_0/Q_0, the

product ee can be greater than the value of 0.95 expected from the E of 39. Hence for this type of reaction, a single value of ee does not give general information about even the initial performance of the enzyme. An even more surprising observation for this type of reaction is illustrated in Fig. 10, where the product ee can actually increases as the reaction proceeds, at least at first. This is observed consistently for somewhat unusual, but not impossible, circumstances with very low concentrations of Q (H_2O) and small values of the enzyme parameter $SCSf/b$.
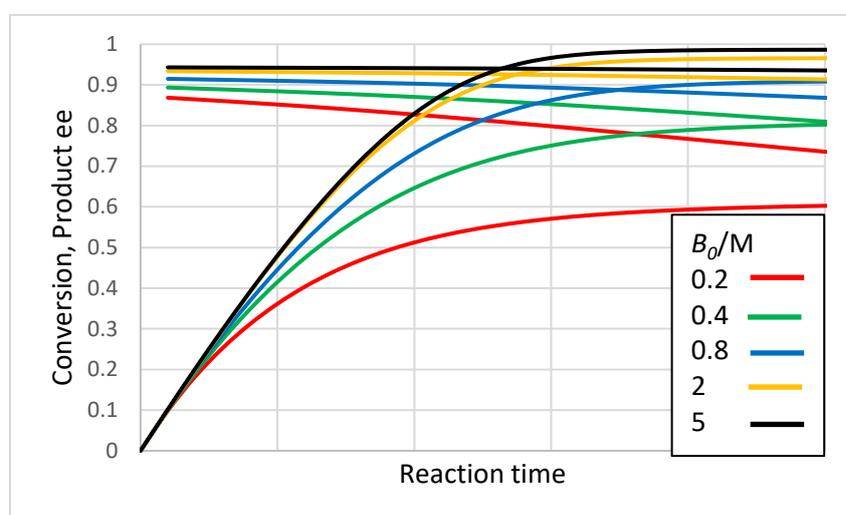
Fig 8. Progress curves for "ping-pong, both" kinetics, diacid esterification. Plot shows increasing conversion of S and decreasing ee of product. Parameters not changed: $S_0 = 0.2$ M, $Q_0 = 0.5$ M, $K_{eq} = 10$, $E = 39$, $K_S = 0.1$ M, $k_{2R} = 1000$ s^{-1}, $SCRf/b = 0.5$, $E2/E = 0.5$, $SCR2f/SCRf = 1$, $SCSf/b = 1$, $k_{4R}/k_{2R} = k_{2R}/E \cdot k_{2S} = k_{4R}/E \cdot k_{4S} = 1$, $k_{-1R}/k_{2R} = k_{-1S}/k_{2S} = k_{-3R}/k_{4R} = k_{-3S}/k_{4S} = 2$. There seems to be no data on K_{eq} for diacid ester reactions, but values range between 2 and 4 for a simple esterification in various non-aqueous solvents [35].
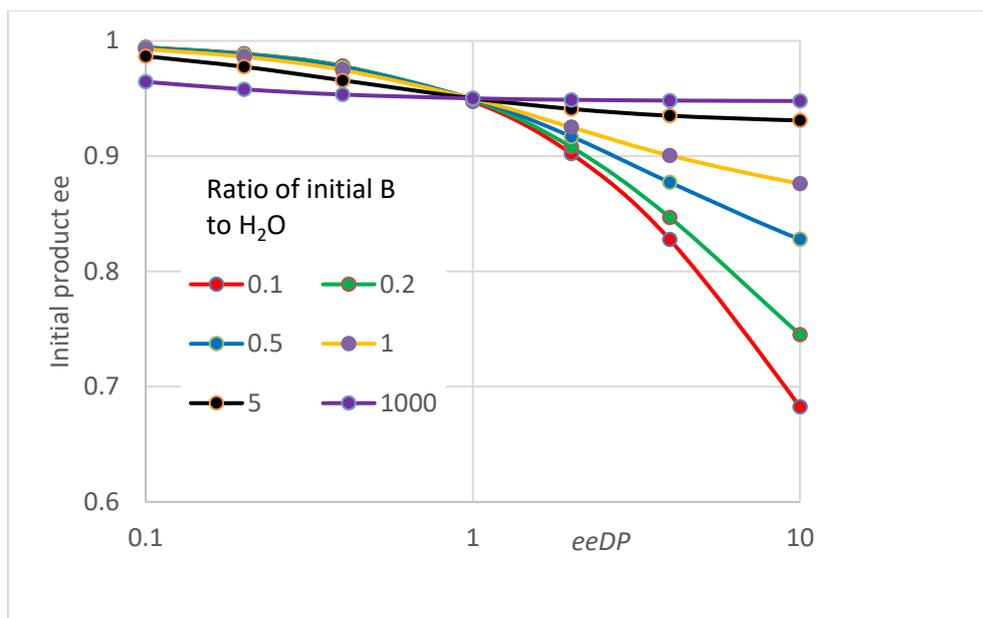
Fig. 9. Effects on ee of product formed early in the reaction for "ping-pong, both" kinetics, diacid esterification. The graph plots ee at a conversion of about 0.1. For this plot the value of *eeDP* was altered by varying *SCSf/b*, but very similar ee values were found if *SCRf/b* or *E2/E* were varied instead. Similarly, Q_0 (the initial H_2O concentration) was varied for the plot, but the ratio B_0/Q_0 is what actually determines the behaviour. Parameters not varied were: S_0 = 0.2 M, B_0 = 0.4 M, K_{eq} = 10, E = 39, K_S = 0.1 M, k_{2R} = 1000 s^{-1}, *SCRf/b* = 0.5, *E2/E* = 0.5, *SCR2f/SCRf* = 1, k_{4R}/k_{2R} = $k_{2R}/E \cdot k_{2S}$ = $k_{4R}/E \cdot k_{4S}$ = 1, k_{-1R}/k_{2R} = k_{-1S}/k_{2S} = k_{-3R}/k_{4R} = k_{-3S}/k_{4S} = 2.
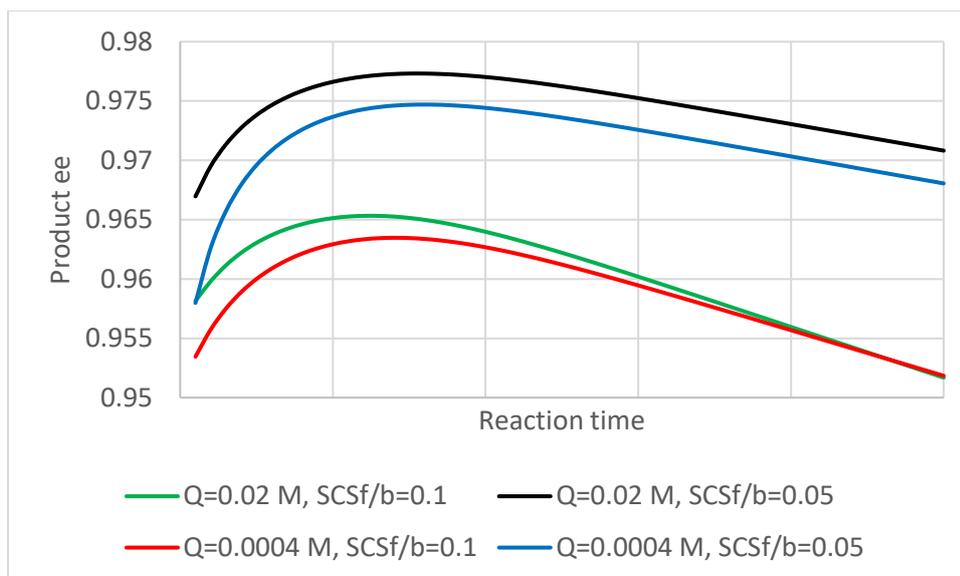
Fig. 10. Increase in product ee as reaction proceeds for "ping-pong, both" kinetics, diacid esterification. There is no point for exactly zero time, because no product has been formed. Other parameters were: S_0 = 0.2 M, B_0 = 0.5 M, K_{eq} = 10, E = 39, K_S = 0.1 M, k_{2R} = 1000 s^{-1}, $SCRf/b$ = 0.5, $E2/E$ = 0.5, $SCR2f/SCRf$ = 1, $k_{4R}/k_{2R} = k_{2R}/E \cdot k_{2S} = k_{4R}/E \cdot k_{4S}$ = 1, $k_{-1R}/k_{2R} = k_{-1S}/k_{2S} = k_{-3R}/k_{4R} = k_{-3S}/k_{4S}$ = 2.. By the end of the reaction time plotted, the conversion is about 0.92, very close to the equilibrium value.

Fig 11 shows effects on the product ee at 95% of the equilibrium conversion, as for the other kinetic mechanisms. Again these ee values can deviate substantially from 0.95 that might be expected for E value of 39 used. And for some parameters, they can actually exceed 0.95, as seen already in the initial rate. The general shape of the plots in part A are similar to those for the initial ee in Fig 9. The plot is again against ee decline parameter ($eeDP$), which is the enzyme property with the most important influence on product ee. However, as shown in part B, in this case there remain important effects of other parameters. Empirical study showed that most of the remaining effect could be accounted for by the value of the product of parameters $SCRf/b$ and $SCR2f/SCRf$. For $eeDP$ > 1, lower values of $SCRf/b \cdot SCR2f/SCRf$ cause a much larger fall in ee as the reaction approaches equilibrium. In

contrast, for *eeDP* < 1, lower values of *SCRf/b·SCR2f/SCRf* actually lead to higher product ee, often greater than 0.95 expected from the *E* of 39. Parameters not varied in Fig. 11 had little or no effect on product ee. It decreased slightly for higher S_0, and increased slightly for higher K_{eq}. There was no noticeable effect of varying K_S, k_{2R}, k_{4R}/k_{2R}, $k_{2R}/E·k_{2S}$, $k_{4R}/E·k_{4S}$, k_{-1R}/k_{2R}, k_{-1S}/k_{2S}, k_{-3R}/k_{4R} or k_{-3S}/k_{4S}.
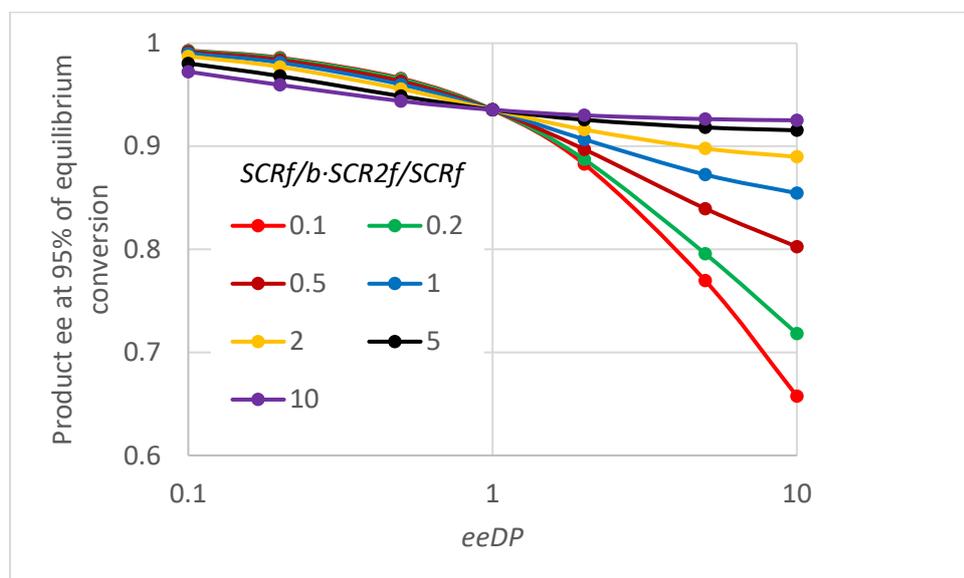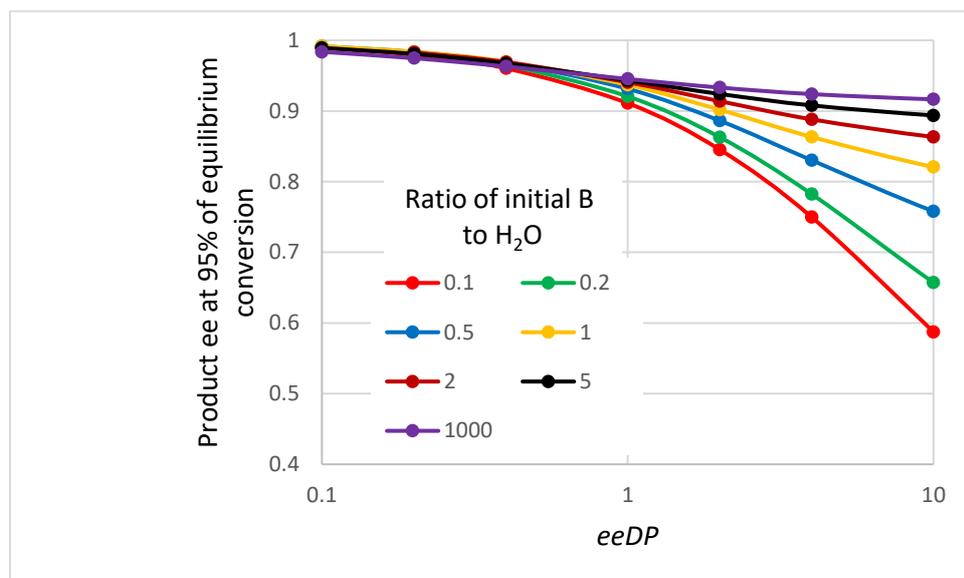
Fig 11. Effects on ee at high conversion for diacid ester synthesis, "ping-pong, both" kinetics.

Parameters not varied were: S_0 = 0.2 M, B_0 = 0.4 M, K_{eq} = 10, E = 39, K_S = 0.1 M, k_{2R} = 1000 s^{-1},

SCR2f/SCRf = 1, $k_{4R}/k_{2R} = k_{2R}/E \cdot k_{2S} = k_{4R}/E \cdot k_{4S} = 1$, $k_{-1R}/k_{2R} = k_{-1S}/k_{2S} = k_{-3R}/k_{4R} = k_{-3S}/k_{4S} = 2$. For part A, the ratio B_0/Q_0 was varied by changing Q_0 (H_2O), although almost identical product ee's were obtained for the same ratio if B_0 was varied instead. Similarly, the ee decline parameter (*eeDP*) was varied by changing *SCSf/b*, with *SCRf/b* = 0.5 and *E2/E* = 0.5, but changing these two parameters gave similar product ee's for any given value of *eeDP*. For part B, Q_0 = 0.5 M, *SCSf/b* = 1. The parameters *SCRf/b* and *E2/E* were both varied, so that each value of *eeDP* could be obtained with different combinations of *SCRf/b* and *E2/E*. Each point would have a different value of the product *SCRf/b·SCR2f/SCRf*. If instead *SCR2f/SCRf* was varied, but this product and *eeDP* were kept constant, the ee values found for the reaction product were very similar.

Expanding the parameters incorporated in *eeDP* and making some cancellations leads to

$$eeDP = \frac{SCSf/b}{\frac{E2}{E} \cdot SCRf/b} = \frac{SCS2f.SCRb}{SCSb.SCR2f}$$

The right hand expression has two interesting ratios of pseudo-specificity constants. *SCS2f/SCSb* governs the relative rate of reaction of the S acyl enzyme with either B to produce S ester product or with Q (H_2O) to give back prochiral diacid. *SCR2f/SCRb* acts the same way for the R acyl enzyme. It makes intuitive sense that such ratios would be important, as formation of the unwanted S product will be favoured if the S acyl enzyme tends to react forward or the R acyl enzyme tends to react backwards. A similar expansion and cancellation from the empirically found product *SCRf/b·SCR2f/SCRf* shows that this is also equal to *SCR2f/SCRb*.

In summary, for this case of diacid ester synthesis, reporting a single product ee as characterising the reaction is particularly misleading. Not only can it vary as the reaction proceeds, but even the initial value can change, including as a result of experimental variables like the initial B (alcohol) concentration.

CONCLUSIONS

The kinetics of enzymatic de-symmetrisation of prochiral starting materials is not as simple as usually assumed. It is a mistake to assume that the product ee will always remain constant throughout the progress of the reaction. The kinetics of many enzymes may lead to product ee declining substantially before reaching preparatively useful conversions. Hence when choosing or modifying enzymes for these reactions it is important to consider the extent to which ee might fall over the time course, as well as its initial value. For the various kinetic mechanisms, it is possible to identify a single enzyme property, an ee decline parameter (*eeDP*), that describes the dominant tendency.

The work indicates that good practice in reporting enzymatic de-symmetrisation experiments would be to state conversion at which an ee value is found. Preferably at least two pairs of conversion and ee should be reported for a given reaction, to show how much the ee tends to decline at higher conversion.

Some effects found could lead to problems in improving a preparative reaction based on initial studies. Clearly a high ee found in initial work at low conversion may be found to decline substantially when reaction time is extended to reach preparative conversions. But the adverse effect (for some kinetic mechanisms) of higher concentrations of prochiral starting material should also be noted. Very often process development will involve increasing these concentrations to try and obtain a more efficient process, but the result may be an undesirable fall in ee at higher conversions.

SUPPORTING INFORMATION

A single file of Supporting Information is available. It contains the Methods section, with full details of how simulations were performed and the mathematical analysis of the various kinetic

mechanisms. It also gives a fuller index of data files (Maple worksheets for the full derivations; MATLAB code files to run the simulations; Excel files containing all calculated progress curves) that can be downloaded via: https://doi.org/10.15129/fbd7e7c0-9712-41a4-a88d-63eecccdd2d9

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SUPPLEMENTARY INFORMATION FOR:

Kinetics of enzyme-catalysed de-symmetrisation of prochiral substrates: product enantiomeric excess is not always constant

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Index to data files available for download

All the following are freely available for download via this link:

https://doi.org/10.15129/fbd7e7c0-9712-41a4-a88d-63eecccdd2d9

Maple worksheets for each of the 4 kinetic models, both in native (.mw) form, and as pdf print-outs.

ProchiralOrdered.mw

ProchiralPing.mw (for "ping-pong, second" kinetics)

ProchiralDiolEsters.mw (for "ping-pong, first" kinetics)

ProchiralDiacids.mw (for "ping-pong, both" kinetics)

MATLAB code files for each of the kinetic models (two for the Diacid case, covering hydrolysis and esterification).

enz_progress_ProchiralOrdered.m

enz_progress_ProchiralPing.m

enz_progress_ProchiralDiol.m

enz_progress_ProchiralDiacid_Hydr.m

enz_progress_ProchiralDiacid_Estfn.m

which call functions defined in separate files:-

ODE_ProchiralOrdered.m

ODE_ProchiralPing.m

ODE_ProchiralDiol.m

ODE_ProchiralDiacid.m

Excel files with full simulated progress data for a wide range of input parameter values.

PlotGraphsOrdered.xlsm

PlotGraphsPing.xlsm

PlotGraphsDiol.xlsm

PlotGraphsDiacid.xlsm

METHODS

Calculation of reaction progress curves by numerical method

Based on the kinetic mechanisms shown in Scheme 1, ordinary differential equations were written

for the change in concentration of all enzyme forms, reactants and products. This set of equations

was then integrated using MATLAB, based on the elementary rate constants, and no analytical

approximations. The in-built ode15s solver was able to handle the integration reasonably quickly

(usually less than 10 s on a 7 years old laptop), despite the equation system being stiff. (Avoiding a

stiff equation set is one reason for the usual approach of using the quasi-steady state

approximation). The tolerance was routinely set at 1E-6; changing to 1E-5 or 1E-7 gave the same

results to at least 1 part in 1000. The differential equations were actually handled in normalised

form without dimensions of concentration:-

all enzyme forms were handled as fractions of the total enzyme concentration (E_0);

S, D, B, Q, PR and PS were all normalised via division by the initial substrate concentration S_0;

and all second order rate constants were multiplied by S_0 to give values with dimensions of time^{-1}.

The ratio of E_0/S_0, typically a small number, appeared as a multiplier for many coefficients in the

differential equations. So for example the fully dimensioned equation:

$$\frac{dS}{dt} = -k_{2R}.ED.S - k_{2S}.ED.S + k_{-2R}.EDS_R + k_{-2S}.EDS_S$$

for the ordered kinetic model is transformed to the normalised form:-

$$\frac{dS'}{dt} = \frac{E_0}{S_0}.(-k'_{2R}.ED'.S' - k'_{2S}.ED'.S' + k_{-2R}.EDS_R' + k_{-2S}.EDS_S')$$

where apostrophes (') indicate normalised values. Using these normalised forms has the effect that

all state variables are of a similar magnitude (typically 0.1 to 10) throughout most of the reaction

progress, which may help with the integration of the model (although this was not tested).

To aid comparison of progress curves for different enzyme properties and initial concentrations, the

ratio E_0/S_0 was set so that the initial rate was always the same. This is essentially equivalent to using

a dimensionless time value. Hence no times are given on the progress curves shown. The necessary

value of E_0/S_0 was calculated using an expression for initial rate obtained through the mathematical analysis of the kinetics (see below).

Driver MATLAB code took values of the appropriate input parameters (see below), calculated the corresponding elementary rate constants, ran the integration, then output values of conversion (total to both product enantiomers) and enantiomeric excess for a series of time points.

All the MATLAB code files used, and all the progress data generated, are freely available for download via the link given at the top of this document.

<div align="center">

Mathematical analysis of kinetics

</div>

As an aid to understanding, and the selection of sensible input enzyme parameters, equations were also solved analytically following the quasi-steady state approximation (setting the rate of change of all enzyme forms to zero). After elimination from the resulting equations (using Maple software to deal with some very large and complicated expressions), it was possible to derive equations for the rate of product formation. Based on this analysis, parameters were selected as inputs for the calculation of progress curves, following these principles:-

   a) values for which separate estimates will often be possible, like K_{eq} and E, the observed ratio of enantiomers (under initial rate conditions);

   b) inspection of the analytical equations for reaction progress, derived using the quasi-steady state approximation;

   and c) empirical observations of highly influential parameters after trials with different possible sets that might be used.

These input parameters were then used to calculate the elementary rate constants used in calculation of progress curves.

The mathematical analysis is now summarised for each kinetic mechanism as defined in Scheme 1 of the main paper, using elementary rate constants for reaction steps shown in that Scheme. The links at the top of this document allow download of all the Maple worksheets, and pdf print-outs of them.

"Ordered, second" kinetics

The set of linked differential equations were solved analytically using the pseudo-steady state approximation that sets the rate of change of enzyme forms to zero. One of the most useful forms for the overall rate equation obtained is the following:

$$\frac{1}{E_0}\frac{dPR}{dt} =$$

$$\frac{\dfrac{D.S.k_1.k_4.k_{2R}.k_{3R}}{k_{3R}+k_{-2R}} + \dfrac{D.S.PS.k_1.k_{2R}.k_{3R}.k_{-2S}.k_{-3S}}{(k_{3R}+k_{-2R}).(k_{3S}+k_{-2S})} + \dfrac{S.Q.PS.k_{2R}.k_{3R}.k_{-2S}.k_{-3S}.k_{-4}}{(k_{3R}+k_{-2R}).(k_{3S}+k_{-2S})}}{\text{denominator}}$$

$$-\frac{PR.Q.k_{-1}.k_{-4}.k_{-2R}.k_{-3R}}{k_{3R}+k_{-2R}} - \frac{PR.D.S.k_1.k_{2S}.k_{3S}.k_{-2R}.k_{-3R}}{(k_{3R}+k_{-2R}).(k_{3S}+k_{-2S})} - \frac{PR.S.Q.k_{2S}.k_{3S}.k_{-2R}.k_{-3R}.k_{-4}}{(k_{3R}+k_{-2R}).(k_{3S}+k_{-2S})}$$

with denominator:

$$k_4.k_{-1} + D.k_1.k_4 + Q.k_{-1}.k_{-4} + S.k_4.\left(\frac{k_{2R}.k_{3R}}{(k_{3R}+k_{-2R})} + \frac{k_{2S}.k_{3S}}{(k_{3S}+k_{-2S})}\right) + \frac{PR.k_{-1}.k_{-2R}.k_{-3R}}{k_{3R}+k_{-2R}}$$

$$+\frac{PS.k_{-1}.k_{-2S}.k_{-3S}}{k_{3S}+k_{-2S}} + D.S.k_1.\left(\frac{k_{2R}.(k_{3R}+k_4)}{(k_{3R}+k_{-2R})} + \frac{k_{2S}.(k_{3S}+k_4)}{(k_{3S}+k_{-2S})}\right) + \frac{D.PR.k_1.k_{-2R}.k_{-3R}}{k_{3R}+k_{-2R}}$$

$$+\frac{D.PS.k_1.k_{-2S}.k_{-3S}}{k_{3S}+k_{-2S}} + S.Q.k_{-4}.\left(\frac{k_{2R}.k_{3R}}{(k_{3R}+k_{-2R})} + \frac{k_{2S}.k_{3S}}{(k_{3S}+k_{-2S})}\right) + \frac{Q.PR.k_{-4}.k_{-3R}.(k_{-1}+k_{-2R})}{(k_{3R}+k_{-2R})}$$

$$+\frac{Q.PS.k_{-4}.k_{-3S}.(k_{-1}+k_{-2S})}{(k_{3S}+k_{-2S})} + D.S.PR.k_1.k_{-3R}.\left(\frac{k_{2S}.(k_{3S}+k_{-2R}) + k_{2R}.(k_{3S}+k_{-2S})}{(k_{3R}+k_{-2R}).(k_{3S}+k_{-2S})}\right)$$

$$+D.S.PS.k_1.k_{-3S}.\left(\frac{k_{2R}.(k_{3R}+k_{-2S}) + k_{2S}.(k_{3R}+k_{-2R})}{(k_{3R}+k_{-2R}).(k_{3S}+k_{-2S})}\right)$$

$$+S.Q.PR.k_{-4}.k_{-3R}.\left(\frac{k_{2S}.(k_{3S}+k_{-2R}) + k_{2R}.(k_{3S}+k_{-2S})}{(k_{3R}+k_{-2R}).(k_{3S}+k_{-2S})}\right)$$

$$+S.Q.PS.k_{-4}.k_{-3S}.\left(\frac{k_{2R}.(k_{3R}+k_{-2S}) + k_{2S}.(k_{3R}+k_{-2R})}{(k_{3R}+k_{-2R}).(k_{3S}+k_{-2S})}\right)$$

Since the derivation makes no assumption about whether the R or S enantiomer is favoured, the corresponding expression for dPS/dt is simply obtained by exchanging every R for S and vice versa in this expression. In fact the appearances of R and S in the denominator are completely symmetrical, so the denominator does not change with this substitution. The expression contains multiple appearances of the sums (k_{3R} + k_{-2R}) and (k_{3S} + k_{-2S}), which arrive naturally from the derivation. Furthermore, (k_{3R} + k_{-2R}) often appears as the denominator in terms with products $k_{2R}.k_{3R}$ or $k_{-2R}.k_{-3R}$ in the numerator, as do the corresponding groups in S. The ratio $k_{2R}.k_{3R}/(k_{3R} + k_{-2R})$ has the form of a pseudo-specificity constant (k_{cat}/K_M) for the reaction of the enzyme form ED with S to produce PR and EQ. Similarly $k_{2S}.k_{3S}/(k_{3S} + k_{-2S})$ is a pseudo-specificity constant for the corresponding reaction producing PS. These pseudo-specificity constants will govern the competition between these two reaction pathways, and hence the ratio of product enantiomers formed. This can also be seen by considering the full expression above under initial rate conditions, when both PR and PS are zero. The ratio of initial rates of PR and PS formation becomes the ratio of the first terms in the numerator for R and S. And this ratio is exactly the ratio of pseudo-specificity constants. The ratios with backward rate constants in the numerator, like $k_{-2R}.k_{-3R}/(k_{3R} + k_{-2R})$, can also be seen as pseudo-specificity constants for the reverse reaction, here of the enzyme form EQ with PR to produce ED and give back S. Hence in understanding the behaviour and simplifying the expression it is useful to define these pseudo-specificity constants as parameters, labelled SC followed by either R or S, then f or b for the forwards or backwards direction.

$$SCRf = \frac{k_{2R}.k_{3R}}{k_{3R}+k_{-2R}}$$

$$SCRb = \frac{k_{-2R}.k_{-3R}}{k_{3R}+k_{-2R}}$$

$$SCSf = \frac{k_{2S}.k_{3S}}{k_{3S} + k_{-2S}}$$

$$SCSb = \frac{k_{-2S}.k_{-3S}}{k_{3S} + k_{-2S}}$$

Substituting these gives the following expression for the rate

$$\frac{dPR}{dt}$$

$$= E_0.\frac{\begin{array}{c} D.S.k_1.k_4.SCRf + D.S.PS.k_1.SCRf.SCSb + S.Q.PS.k_{-4}.SCRf.SCSb \\ -PR.Q.k_{-1}.k_{-4}.SCRb - PR.D.S.k_1.SCSf.SCRb - PR.S.Q.k_{-4}.SCSf.SCRb \end{array}}{\begin{array}{c} k_4.k_{-1} + D.k_1.k_4 + Q.k_{-1}.k_{-4} + S.k_4.(SCRf + SCSf) + PR.k_{-1}.SCRb \\[4pt] +PS.k_{-1}.SCSb + D.S.k_1.\left(SCRf.\left(1 + \frac{k_4}{k_{3R}}\right) + SCSf.\left(1 + \frac{k_4}{k_{3S}}\right)\right) + D.PR.k_1.SCRb \\[4pt] +D.PS.k_1.SCSb + S.Q.k_{-4}.(SCRf + SCSf) + Q.PR.k_{-4}.SCRb.\left(1 + \frac{k_{-1}}{k_{-2R}}\right) \\[4pt] +Q.PS.k_{-4}.SCSb.\left(1 + \frac{k_{-1}}{k_{-2S}}\right) + D.S.PR.k_1.SCRb.\left(\frac{SCSf}{k_{-2R}} + \frac{SCSf}{k_{3S}} + \frac{k_{2R}}{k_{-2R}}\right) \\[4pt] +D.S.PS.k_1.SCSb.\left(\frac{SCRf}{k_{-2S}} + \frac{SCRf}{k_{3R}} + \frac{k_{2S}}{k_{-2S}}\right) + S.Q.PR.k_{-4}.SCRb.\left(\frac{SCSf}{k_{-2R}} + \frac{SCSf}{k_{3S}} + \frac{k_{2R}}{k_{-2R}}\right) \\[4pt] +S.Q.PS.k_{-4}.SCSb.\left(\frac{SCRf}{k_{-2S}} + \frac{SCRf}{k_{3R}} + \frac{k_{2S}}{k_{-2S}}\right) \end{array}}$$

It should be noted that now k_{-3R} and k_{-3S} no longer appear as separate parameters.

Under initial rate conditions with Q, PS and PR all zero, the expression reduces to

$$\frac{dPR}{dt} = \frac{E_0.D.S.SCRf}{\frac{k_{-1}}{k_1} + D + S.\left(\frac{SCRf + SCSf}{k_1}\right) + D.S.\left(\frac{SCRf}{k_4} + \frac{SCSf}{k_4} + \frac{SCRf}{k_{3R}} + \frac{SCSf}{k_{3S}}\right)}$$

Comparison with the usual expression for the initial rate of an enzyme following the ordered mechanism gives the K_M values for substrates D and S as:

$$K_{MS} = \frac{1}{\left(\frac{SCRf}{k_4} + \frac{SCSf}{k_4} + \frac{SCRf}{k_{3R}} + \frac{SCSf}{k_{3S}}\right)}$$

$$K_{MD} = \frac{\left(\frac{SCRf + SCSf}{k_1}\right)}{\left(\frac{SCRf}{k_4} + \frac{SCSf}{k_4} + \frac{SCRf}{k_{3R}} + \frac{SCSf}{k_{3S}}\right)}$$

In choosing parameters to define the behaviour, it is sensible to include ones that are quite likely to be known or at least be estimated. Hence they include K_{eq} for the overall reaction, and E for the observed ratio of enantiomers (under initial rate conditions). As shown above, E = SCRf/SCSf. Then use K_{MD} and K_{MS}, which will be used in the relationships immediately above. The rate constant arbitrarily fixed to set the overall timescale can be chosen as k_4. Two further parameters selected are the ratios k_{3R}/k_4 (which does appear in the full rate expression) and $k_{3R}/(E.k_{3S})$. Both these parameters are dimensionless, and are expected to be of the order of magnitude of 1 (E is

introduced in the definition of the second parameter to bring it closer to 1). Using the values of k_4, k_{3R}/k_4, $k_{3R}/(E.k_{3S})$, E and K_{MS}, the equation above allows calculation of SCRf and SCRb. Then the value of K_{MD} allows calculation of k_1.

The dimensionless ratio SCRf/b = SCRf/SCRb affects the relative magnitude of terms in the full rate expression, and was also found empirically to have a key influence on behaviour. Its introduction allows calculation of SCRb. Now make use of the equilibrium constant for racemisation of the product, which must equal 1, and can be related to SC values, allowing calculation of SCSb:

$$K_{rac} = 1 = \frac{k_{2R}.k_{3R}.k_{-2S}.k_{-3S}.}{k_{2S}.k_{3S}.k_{-2R}.k_{-3R}.} = \frac{SCRf.SCSb}{SCSf.SCRb}$$

Now introduce the ratio k_{-4}/k_1. This also controls the relative magnitude of many terms in the full rate expression; dividing this expression top and bottom by one of these rate constants will generate many examples of k_{-4}/k_1. Setting this ratio as a parameter allows the calculation of k_{-4}. Then make use of the equilibrium constant K_{eq} for formation of PR and Q from D and S, which is related to rate constants by

$$K_{eq} = \frac{k_1.k_{2R}.k_{3R}.k_4}{k_{-1}.k_{-2R}.k_{-3R}.k_{-4}} = \frac{k_1.k_4.SCRf}{k_{-1}.k_{-4}.SCRb}$$

which allows calculation of k_{-1}.

Finally introduce two more ratios k_{-2R}/k_{-1} and $E.k_{-2S}/k_{-1}$. Both these ratios of rate constants appear in the full rate expression, and they are both first order rate constants so the ratio is dimensionless. Their values are again likely to be of the order of magnitude of 1. Using k_{-2R}/k_{-1} and $E.k_{-2S}/k_{-1}$, the values of k_{-2R} and k_{-2S} can be calculated, and hence all remaining rate constants using the known values of SC's.

To aid comparison of progress simulations, it is desirable that they all have the same initial rate. In the case of ordered kinetics, it is assumed that cofactor recycling will be used, so the conditions are approximated as having constant non-zero concentrations of both D and Q (e.g. NADH and NAD$^+$). Under these conditions the full rate expression reduces to:

$$\frac{1}{S_0}\frac{dPR}{dt} = \frac{E_0.D.k_1.k_4.SCRf}{\begin{array}{c} k_4.k_{-1} + D.k_1.k_4 + Q.k_{-1}.k_{-4} + S_0.k_4.(SCRf + SCSf) \\ + D.S_0.k_1.\left(SCRf.\left(1 + \frac{k_4}{k_{3R}}\right) + SCSf.\left(1 + \frac{k_4}{k_{3S}}\right)\right) + S_0.Q.k_{-4}.(SCRf + SCSf) \end{array}}$$

Where S_0 is the initial concentration of S and $(1/S_0).(dPR/dt)$ is the dimensionless initial rate of conversion to PR. There will also be an initial rate of conversion to PS, but because of the symmetry in the expressions, this will be a fixed fraction of that to PR, for any given E value. Hence the rate of increase in overall conversion will be higher by a factor of $(1 + 1/E)$. Assuming the R enantiomer is favoured, this factor will be slightly greater than 1; if the S enantiomer is favoured, it is probably best to exchange R and S in the expressions above, such that E is again defined such that it is greater than 1.

"Ping-pong, second" kinetics

In this case the usual pseudo-steady state approximation leads to the following full expression for the rate of product formation:-

$$\frac{dPR}{dt}$$

$$= E_0 \cdot \frac{\frac{D.S.k_1.k_2.k_{3R}.k_{4R}}{(k_{4R}+k_{-3R})}+\frac{S.PS.k_{3R}.k_{-3S}.k_{-4S}.(k_2+k_{-1})}{(k_{4S}+k_{-3S})}-\frac{PR.Q.k_{-1}.k_{-2}.k_{-3R}.k_{-4R}}{(k_{4R}+k_{-3R})}-\frac{PR.S.k_{-3R}.(k_2+k_{-1}).(k_{3S}.k_{4S}.k_{-4R}+k_{3R}.k_{-3S}.k_{-4S})}{(k_{4R}+k_{-3R}).(k_{4S}+k_{-3S})}}{\begin{array}{l}D.k_1.k_2+S.(k_2+k_{-1}).\left(\frac{k_{3R}.k_{4R}}{(k_{4R}+k_{-3R})}+\frac{k_{3S}.k_{4S}}{(k_{4S}+k_{-3S})}\right)+Q.k_{-1}.k_{-2}+\frac{PR.k_{-3R}.k_{-4R}.(k_2+k_{-1})}{(k_{4R}+k_{-3R})}\\[2mm]+\frac{PS.k_{-3S}.k_{-4S}.(k_2+k_{-1})}{(k_{4S}+k_{-3S})}+D.S.k_1.\left(\frac{k_{3R}.(k_2+k_{4R})}{(k_{4R}+k_{-3R})}+\frac{k_{3S}.(k_2+k_{4S})}{(k_{4S}+k_{-3S})}\right)+D.Q.k_1.k_{-2}\\[2mm]+\frac{PR.S.k_{-4R}.(k_2+k_{-1}).\left(k_{3R}.(k_{4S}+k_{-3S})+k_{3S}.(k_{4S}+k_{-3R})\right)}{(k_{4R}+k_{-3R}).(k_{4S}+k_{-3S})}\\[2mm]+\frac{PS.S.k_{-4S}.(k_2+k_{-1}).\left(k_{3S}.(k_{4R}+k_{-3R})+k_{3R}.(k_{4R}+k_{-3S})\right)}{(k_{4R}+k_{-3R}).(k_{4S}+k_{-3S})}\\[2mm]+\frac{PR.Q.k_{-2}.k_{-4R}.(k_{-1}+k_{-3R})}{(k_{4R}+k_{-3R})}+\frac{PS.Q.k_{-2}.k_{-4S}.(k_{-1}+k_{-3S})}{(k_{4S}+k_{-3S})}\end{array}}$$

As before, exchanging every R for S and vice versa gives an expression for dPS/dt (the denominator is in fact again unchanged). Here the sums $(k_{4R}+k_{-3R})$ and $(k_{4S}+k_{-3S})$ appear frequently, often as denominators with products like $k_{3R}.k_{4R}$ or $k_{-3R}.k_{-4R}$ in the numerator. So there are similar pseudo-specificity constants like $k_{3R}.k_{4R}/(k_{4R}+k_{-3R})$, this one for the reaction of the substituted enzyme E* with S to produce PR and the free enzyme E. Again as before, under initial rate conditions when PR, PS and Q are zero, the equation shows that the ratio of enantiomers formed is equal to the ratio of forward pseudo-specificity constants for R and S. Hence we define:

$$SCRf = \frac{k_{3R}.k_{4R}}{k_{4R}+k_{-3R}}$$

$$SCRb = \frac{k_{-3R}.k_{-4R}}{k_{4R}+k_{-3R}}$$

$$SCSf = \frac{k_{3S}.k_{4S}}{k_{4S}+k_{-3S}}$$

$$SCSb = \frac{k_{-3S}.k_{-4S}}{k_{4S}+k_{-3S}}$$

The backward constants SCRb and SCSb here are pseudo-specificity constants for the part reaction of the free enzyme E with PR or PS to produce S and substituted enzyme E*. The full rate expression also contains several appearances of the term (k_2+k_{-1}), in a ratio with $k_1.k_2$. Hence it is appropriate to define also a pseudo-specificity constant for the achiral first part reaction of enzyme E with D to produce Q and substituted enzyme E*.

$$SCD = \frac{k_1.k_2}{k_2 + k_{-1}}$$

By substituting all 5 of these pseudo-specificity constants we obtain the following rate expression:

$$\frac{dPR}{dt}$$

$$= E_0 . \frac{D.S.SCRf + \dfrac{S.PS.k_{3R}.SCSb}{SCD} - \dfrac{PR.Q.k_{-1}.k_{-2}.SCRb}{k_1.k_2} - \dfrac{PR.S.SCRb}{SCD}\left(SCSf + \dfrac{k_{3R}.SCSb}{k_{-4R}}\right)}{\begin{array}{c} D + S.\dfrac{SCRf + SCSf}{SCD} + \dfrac{Q.k_{-1}.k_{-2}}{k_1.k_2} + \dfrac{PR.SCRb}{SCD} + \dfrac{PS.SCSb}{SCD} \\[2mm] + D.S.\left(\dfrac{SCRf.(k_2 + k_{4R})}{k_2.k_{4R}} + \dfrac{SCSf.(k_2 + k_{4S})}{k_2.k_{4S}}\right) + \dfrac{D.Q.k_{-2}}{k_2} \\[2mm] + PR.S.\dfrac{SCRb}{SCD}.\left(\dfrac{SCSf}{k_{-3R}} + \dfrac{SCSf}{k_{4S}} + \dfrac{k_{3R}}{k_{-3R}}\right) + PS.S.\dfrac{SCSb}{SCD}.\left(\dfrac{SCRf}{k_{-3S}} + \dfrac{SCRf}{k_{4R}} + \dfrac{k_{3S}}{k_{-3S}}\right) \\[2mm] + \dfrac{PR.Q.SCRb.k_{-2}.(k_{-1} + k_{-3R})}{k_1.k_2.k_{-3R}} + \dfrac{PS.Q.SCSb.k_{-2}.(k_{-1} + k_{-3S})}{k_1.k_2.k_{-3S}} \end{array}}$$

The rate constant k_{-4S} does not now appear separately. Other rate constants only appear in particular groups: k_1 as $k_1.k_2/k_{-2}$; k_{-1} as $k_{-1}/(k_1.k_2)$; k_{-2} as k_{-2}/k_2; k_{-4R} as k_{-4R}/k_{3R}.

Under initial rate conditions with Q, PR and PS all zero, this reduces to:

$$\frac{dPR}{dt} = \frac{E_0.D.S.SCRf}{D + S.\left(\dfrac{SCRf}{SCD} + \dfrac{SCSf}{SCD}\right) + D.S.\left(\dfrac{SCRf}{k_{4R}} + \dfrac{SCRf}{k_2} + \dfrac{SCSf}{k_{4S}} + \dfrac{SCSf}{k_2}\right)}$$

Comparison with the standard equation for Ping-Pong kinetics shows that the K_M values for substrates D and S are equal to:

$$K_{MS} = \frac{1}{\left(\dfrac{SCRf}{k_{4R}} + \dfrac{SCRf}{k_2} + \dfrac{SCSf}{k_{4S}} + \dfrac{SCSf}{k_2}\right)}$$

$$K_{MD} = \frac{\left(\dfrac{SCRf + SCSf}{SCD}\right)}{\left(\dfrac{SCRf}{k_{4R}} + \dfrac{SCRf}{k_2} + \dfrac{SCSf}{k_{4S}} + \dfrac{SCSf}{k_2}\right)}$$

The initial rate equation can also be used to calculate the ratio of E_0 to S_0 required to achieve a desired initial rate of increase in conversion, as used in comparing the progress for different parameter values.

Parameters that are defined as inputs to examine model behaviour include K_{eq}, enantiomer ratio E (= SCRf/SCSf), K_{MS} and K_{MD}. Then k_2 is included as the rate constant fixed to set the timescale. Ratios of rate constants k_2/k_{4R} and $E.k_{4S}/k_{4R}$ are used as parameters that are dimensionless and expected to be of the order of magnitude of 1, and have some support from the overall rate expression. They allow calculation of SCRf from K_{MS}, and then SCD using K_{MD} as well.

The parameter SCRf/b (= SCRf/SCRb) is dimensionless, and was found empirically to be very influential on relative formation of the two enantiomers. It may tend to be larger for reactions with a

larger overall K_{eq}, but the relationship will not be simple. For example, using an activated acyl donor in a transesterification reaction will raise K_{eq} via effects on k_1, k_2, k_{-1} and k_{-2}. The second part reaction and hence SCRf/b will not be affected. We now use the relationship for equilibration of the two product enantiomers:

$$K_{rac} = 1 = \frac{k_{3R}.k_{4R}.k_{-3S}.k_{-4S}.}{k_{3S}.k_{4S}.k_{-3R}.k_{-4R}.} = \frac{SCRf.SCSb}{SCSf.SCRb}$$

which allows calculation of SCSb.

Now introduce the ratio k_{-1}/k_2, which accounts for all appearances of k_{-1} in the full rate expression, and allows calculation of k_{-1}. Then make use of the equilibrium constant K_{eq} for formation of PR and Q from D and S, which is related to rate constants by

$$K_{eq} = \frac{k_1.k_2.k_{3R}.k_{4R}}{k_{-1}.k_{-2}.k_{-3R}.k_{-4R}} = \frac{k_1.k_2.SCRf}{k_{-1}.k_{-2}.SCRb} = \frac{SCD.(k_2+k_{-1}).SCRf}{k_{-1}.k_{-2}.SCRb}$$

which allows calculation of k_{-2}. Then using the definition of SCD we obtain k_1.

The final two input parameters are defined as the ratios k_{3R}/k_{-4R} and k_{3S}/k_{-4S}. These are dimensionless, and account for the only appearance of k_{-4R} in the overall rate expression (k_{-4S} does not appear at all). There may again be a tendency for these parameters to increase with K_{eq}, but this will certainly not be automatic, as explained for SCRf/b. Together with the known values of SCRf, SCRb, SCSf and SCSb, these allow calculation of all remaining rate constants.

"Ping-pong, first" kinetics: Diol ester hydrolysis

The most likely occurrence of this kinetic mechanism is for hydrolysis of the diesters of prochiral diols. In such cases, the water concentration in the reaction mixture will always be in large excess and hence essentially constant. Thus we use a pseudo-first order rate constant k_{3w} ($=k_3.[H_2O]$) instead of the second order rate constant k_3. The pseudo-steady state approximation then leads to the following full rate expression:-

$$\frac{1}{E_0}\frac{dPR}{dt}$$

$$=\frac{\dfrac{S.k_{1R}.k_{2R}.k_{3w}.k_4}{(k_{2R}+k_{-1R})}+\dfrac{S.PS.k_{1R}.k_{2R}.k_{-1S}.k_{-2S}.(k_4+k_{-3})}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S})}-\dfrac{PR.Q.k_{-1R}.k_{-2R}.k_{-3}.k_{-4}}{(k_{2R}+k_{-1R})}-\dfrac{PR.S.k_{1S}.k_{2S}.k_{-1R}.k_{-2R}.(k_4+k_{-3})}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S})}}{\begin{aligned}&k_{3w}.k_4+S.\left(\frac{k_{1R}.[k_{2R}.(k_4+k_{-3})+k_{3w}.(k_{2R}+k_4)]}{(k_{2R}+k_{-1R})}+\frac{k_{1S}.[k_{2S}.(k_4+k_{-3})+k_{3w}.(k_{2S}+k_4)]}{(k_{2S}+k_{-1S})}\right)\\[4pt]&+Q.k_{-4}.(k_{3w}+k_{-3})+\frac{PR.k_{-1R}.k_{-2R}.(k_4+k_{-3})}{k_{2R}+k_{-1R}}+\frac{PS.k_{-1S}.k_{-2S}.(k_4+k_{-3})}{k_{2S}+k_{-1S}}\\[4pt]&+S.PR.k_{-2R}.(k_4+k_{-3}).\left(\frac{k_{1R}.(k_{2S}+k_{-1S})+k_{1S}.(k_{2S}+k_{-1R})}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S})}\right)\\[4pt]&+S.PS.k_{-2S}.(k_4+k_{-3}).\left(\frac{k_{1S}.(k_{2R}+k_{-1R})+k_{1R}.(k_{2R}+k_{-1S})}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S})}\right)\\[4pt]&+\frac{Q.PR.k_{-2R}.k_{-4}.(k_{-1R}+k_{-3})}{(k_{2R}+k_{-1R})}+\frac{Q.PS.k_{-2S}.k_{-4}.(k_{-1S}+k_{-3})}{(k_{2S}+k_{-1S})}\end{aligned}}$$

As before replacing every R with S and vice versa gives the expression for the rate of PS formation. Again as before the denominator is completely symmetrical and so unchanged on such a replacement. The frequent occurrence of sums (k_{2R} + k_{-1R}) and (k_{2S} + k_{-1S}), usually as divisors to products of rate constants, suggests again the definition of pseudo-specificity constants for the first half reaction of the enzyme with the diol diester S to produce monoester Q and the acyl enzyme intermediate E*.

$$SCRf=\frac{k_{1R}.k_{2R}}{k_{2R}+k_{-1R}}$$

$$SCRb=\frac{k_{-1R}.k_{-2R}}{k_{2R}+k_{-1R}}$$

$$SCSf=\frac{k_{1S}.k_{2S}}{k_{2S}+k_{-1S}}$$

$$SCSb=\frac{k_{-1S}.k_{-2S}}{k_{2S}+k_{-1S}}$$

Substituting these leads to the following expression:

$$\frac{dPR}{dt}=E_0.\frac{S.\dfrac{SCRf.k_{3w}.k_4}{k_4+k_{-3}}+S.PS.SCRf.SCSb-PR.Q.\dfrac{SCRb.k_{-3}.k_{-4}}{k_4+k_{-3}}-PR.S.SCRb.SCSf}{\begin{aligned}&\frac{k_{3w}.k_4}{k_4+k_{-3}}+S.\left(SCRf.\left[1+\frac{k_{3w}}{k_4+k_{-3}}.\left(1+\frac{k_4}{k_{2R}}\right)\right]+SCSf.\left[1+\frac{k_{3w}}{k_4+k_{-3}}.\left(1+\frac{k_4}{k_{2S}}\right)\right]\right)\\[4pt]&+Q.\frac{k_{-4}.(k_{3w}+k_{-3})}{k_4+k_{-3}}+PR.SCRb+PS.SCSb+\\[4pt]&+S.PR.\left(\frac{SCRb.SCSf}{k_{2S}}+\frac{SCRb.SCSf}{k_{-1R}}+\frac{k_{-2R}.SCRf}{k_{2R}}\right)\\[4pt]&+S.PS.\left(\frac{SCRf.SCSb}{k_{2R}}+\frac{SCRf.SCSb}{k_{-1S}}+\frac{k_{-2S}.SCSf}{k_{2S}}\right)\\[4pt]&+Q.PR.\frac{SCRb.k_{-4}}{(k_4+k_{-3})}.\left(1+\frac{k_{-3}}{k_{-1R}}\right)+Q.PS.\frac{SCSb.k_{-4}}{(k_4+k_{-3})}.\left(1+\frac{k_{-3}}{k_{-1S}}\right)\end{aligned}}$$

The rate constants k_{1R} and k_{1S} no longer appear individually in this expression.

Under initial rate conditions with Q, PR and PS all zero, the expression reduces to:

$$\frac{dPR}{dt} = \frac{E_0.S.SCRf.k_{3w}.k_4}{k_{3w}.k_4 + S.\left(SCRf.\left[k_4 + k_{-3} + k_{3w}.\left(1 + \frac{k_4}{k_{2R}}\right)\right] + SCSf.\left[k_4 + k_{-3} + k_{3w}.\left(1 + \frac{k_4}{k_{2S}}\right)\right]\right)}$$

The denominator is unchanged on exchanging R and S, so the initial rates of formation of the two enantiomers are once again in the ratio SCRf/SCSf, i.e. E. From this initial rate expression it is also clear that the K_M value (for S) is given by:

$$K_M = \frac{k_{3w}.k_4}{\left(SCRf.\left[k_4 + k_{-3} + k_{3w}.\left(1 + \frac{k_4}{k_{2R}}\right)\right] + SCSf.\left[k_4 + k_{-3} + k_{3w}.\left(1 + \frac{k_4}{k_{2S}}\right)\right]\right)}$$

As input parameters we again use the initial enantiomer ratio E (= SCRf/SCSf), the K_M value (for S), and the overall equilibrium constant K_{eq}. (Note that for this kinetic model, K_{eq} has dimensions, with units taken as M). Choose k_4 as the rate constant given a value to set the timescale.

To proceed, define as input parameters two ratios of rate constants, k_{2R}/k_4 and $k_{2R}/(E.k_{2S})$. Both these are dimensionless, and are expected to be of the order of magnitude of 1. The ratio k_{2R}/k_4 also appears in the overall rate expression. Define also k_{3w}/k_4, which is dimensionless, likely to be of the order of magnitude of 1, and has some support from the overall rate expression.

The ratio k_{-4}/k_4 also has some support from the overall rate expression, but has dimensions of M^{-1}. Hence we actually define the input parameter as $k_{-4}.K_{eq}/k_4$, which is dimensionless. It will often be of the order of 1, but could deviate significantly depending on whether the first or second stage of the reaction dominates the overall equilibrium. The ratio SCRf/b (=SCRf/SCRb) is again found empirically to be very important in determining the behaviour. Then make use of the following relationship of the overall equilibrium constant

$$K_{eq} = \frac{k_{1R}.k_{2R}.k_{3w}.k_4}{k_{-1R}.k_{-2R}.k_{-3}.k_{-4}} = \frac{SCRf.k_{3w}.k_4}{SCRb.k_{-3}.k_{-4}}$$

Now using K_{eq}, k_4, k_{3w}/k_4, $k_{-4}.K_{eq}/k_4$ and SCRf/b, we can calculate k_{3w}, k_{-4} and then k_{-3}. Then using k_{2R}/k_4, $k_{2R}/(E.k_{2S})$, E and K_M, we can calculate k_{2R}, k_{2S} and then SCRf and SCSf (the latter using the expression above for K_M).

For equilibration of the product enantiomers we have

$$K_{rac} = 1 = \frac{k_{1R}.k_{2R}.k_{-1S}.k_{-2S}.}{k_{1S}.k_{2S}.k_{-1R}.k_{-2R}.} = \frac{SCRf.SCSb}{SCSf.SCRb}$$

Hence using SCRf/b again we obtain SCSb.

Finally, define two more ratios of rate constants, k_{-1R}/k_{-3} and $E.k_{-1S}/k_{-3}$, which appear in the overall rate expression. These are dimensionless, and may be of the order of 1, but a very different value is

also possible. With the values of 4 SC's and 2 rate constants for the first part reaction, these two final parameters allow calculation of all remaining rate constants.

"Ping-pong, both" kinetics: Diacid case

For this kinetic model there are no less than 16 separate elementary rate constants, and 8 different enzyme forms. Using the quasi-steady state approximation it is possible to derive the following expression for the rate of product formation:-

$$
\frac{1}{E_0}\frac{dPR}{dt}
$$

$$
= \frac{
\begin{array}{c}
\dfrac{S.B^2.k_{1R}.k_{2R}.k_{3R}.k_{4R}.k_{3S}.k_{4S}}{(k_{2R}+k_{-1R}).(k_{4R}+k_{-3R}).(k_{4S}+k_{-3S})} + \dfrac{S.B.Q.k_{1R}.k_{2R}.k_{3R}.k_{4R}.k_{-1S}.k_{-2S}}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S}).(k_{4R}+k_{-3R})} \\[2em]
- \dfrac{PR.B.Q.k_{3S}.k_{4S}.k_{-1R}.k_{-2R}.k_{-3R}.k_{-4R}}{(k_{2R}+k_{-1R}).(k_{4R}+k_{-3R}).(k_{4S}+k_{-3S})} - \dfrac{PR.Q^2.k_{-1R}.k_{-2R}.k_{-3R}.k_{-4R}.k_{-1S}.k_{-2S}}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S}).(k_{4R}+k_{-3R})}
\end{array}
}{
\begin{array}{c}
S.B.\left(\dfrac{k_{1R}.k_{2R}.k_{3S}.k_{4S}}{(k_{2R}+k_{-1R}).(k_{4S}+k_{-3S})} + \dfrac{k_{1S}.k_{2S}.k_{3R}.k_{4R}}{(k_{2S}+k_{-1S}).(k_{4R}+k_{-3R})}\right) \\[1.5em]
+ \dfrac{S.B^2.k_{3R}.k_{3S}}{(k_{4R}+k_{-3R}).(k_{4S}+k_{-3S})}.\left(\dfrac{k_{1R}.k_{4S}.(k_{2R}+k_{4R})}{(k_{2R}+k_{-1R})} + \dfrac{k_{1S}.k_{4R}.(k_{2S}+k_{4S})}{(k_{2S}+k_{-1S})}\right) \\[1.5em]
+S.Q.\dfrac{k_{1R}.k_{2R}.k_{-1S}.k_{-2S}+k_{1S}.k_{2S}.k_{-1R}.k_{-2R}}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S})} + S.Q^2.\dfrac{k_{-2R}.k_{-2S}.(k_{1R}.k_{-1S}+k_{1S}.k_{-1R})}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S})} \\[1.5em]
+S.B.Q.\left(\begin{array}{c}\dfrac{k_{3R}.k_{-2S}.[k_{1R}.k_{-1S}.(k_{2R}+k_{4R})+k_{1S}.k_{4R}.(k_{2R}+k_{-1R})]}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S}).(k_{4R}+k_{-3R})} \\[1.2em] +\dfrac{k_{3S}.k_{-2R}.[k_{1S}.k_{-1R}.(k_{2S}+k_{4S})+k_{1R}.k_{4S}.(k_{2S}+k_{-1S})]}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S}).(k_{4S}+k_{-3S})}\end{array}\right) \\[2.5em]
\dfrac{B^2.k_{3R}.k_{4R}.k_{3S}.k_{4S}}{(k_{4R}+k_{-3R}).(k_{4S}+k_{-3S})} + \dfrac{Q^2.k_{-1R}.k_{-2R}.k_{-1S}.k_{-2S}}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S})} \\[1.5em]
+B.Q.\left(\dfrac{k_{3R}.k_{4R}.k_{-1S}.k_{-2S}}{(k_{2S}+k_{-1S}).(k_{4R}+k_{-3R})} + \dfrac{k_{3S}.k_{4S}.k_{-1R}.k_{-2R}}{(k_{2R}+k_{-1R}).(k_{4S}+k_{-3S})}\right) \\[1.5em]
+\dfrac{PR.B.k_{3S}.k_{4S}.k_{-3R}.k_{-4R}}{(k_{4R}+k_{-3R}).(k_{4S}+k_{-3S})} + \dfrac{PS.B.k_{3R}.k_{4R}.k_{-3S}.k_{-4S}}{(k_{4R}+k_{-3R}).(k_{4S}+k_{-3S})} \\[1.5em]
+\dfrac{PR.B^2.k_{3R}.k_{3S}.k_{4S}.k_{-4R}}{(k_{4R}+k_{-3R}).(k_{4S}+k_{-3S})} + \dfrac{PS.B^2.k_{3S}.k_{3R}.k_{4R}.k_{-4S}}{(k_{4R}+k_{-3R}).(k_{4S}+k_{-3S})} \\[1.5em]
+\dfrac{PR.Q.k_{-3R}.k_{-4R}.k_{-1S}.k_{-2S}}{(k_{2S}+k_{-1S}).(k_{4R}+k_{-3R})} + \dfrac{PS.Q.k_{-3S}.k_{-4S}.k_{-1R}.k_{-2R}}{(k_{2R}+k_{-1R}).(k_{4S}+k_{-3S})} \\[1.5em]
+\dfrac{PR.Q^2.k_{-1S}.k_{-2S}.k_{-2R}.k_{-4R}.(k_{-1R}+k_{-3R})}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S}).(k_{4R}+k_{-3R})} + \dfrac{PS.Q^2.k_{-1R}.k_{-2R}.k_{-2S}.k_{-4S}.(k_{-1S}+k_{-3S})}{(k_{2R}+k_{-1R}).(k_{2S}+k_{-1S}).(k_{4S}+k_{-3S})} \\[1.5em]
+\dfrac{PR.B.Q.k_{-4R}}{(k_{4R}+k_{-3R})}.\left(\dfrac{k_{3R}.k_{-1S}.k_{-2S}}{(k_{2S}+k_{-1S})} + \dfrac{k_{3S}.k_{4S}.k_{-2R}.(k_{-1R}+k_{-3R})}{(k_{2R}+k_{-1R}).(k_{4S}+k_{-3S})}\right) \\[1.5em]
+\dfrac{PS.B.Q.k_{-4S}}{(k_{4S}+k_{-3S})}.\left(\dfrac{k_{3S}.k_{-1R}.k_{-2R}}{(k_{2R}+k_{-1R})} + \dfrac{k_{3R}.k_{4R}.k_{-2S}.(k_{-1S}+k_{-3S})}{(k_{2S}+k_{-1S}).(k_{4R}+k_{-3R})}\right)
\end{array}
}
$$

As with the diol ester hydrolysis case, this expression contains multiple occurrences of the groups $(k_{2R}+k_{-1R})$ and $(k_{2S}+k_{-1S})$. (Their total number could be reduced slightly by cancellation top and bottom, but they are retained as shown here because of the next step.) They appear with products of rate constants that suggest the definition of pseudo-specificity constants as before:

$$SCRf = \frac{k_{1R}.k_{2R}}{k_{2R} + k_{-1R}}$$

$$SCRb = \frac{k_{-1R}.k_{-2R}}{k_{2R} + k_{-1R}}$$

$$SCSf = \frac{k_{1S}.k_{2S}}{k_{2S} + k_{-1S}}$$

$$SCSb = \frac{k_{-1S}.k_{-2S}}{k_{2S} + k_{-1S}}$$

But in this case there are also frequent occurrences of (k_{4R} + k_{-3R}) and (k_{4S} + k_{-3S}). These naturally suggest a further set of pseudo-specificity constants, which apply to the second stage part reaction converting the substituted enzyme E* (acyl enzyme intermediate) back to the free enzyme, liberating either the free acid or the ester. These are defined as follows:

$$SCR2f = \frac{k_{3R}.k_{4R}}{k_{4R} + k_{-3R}}$$

$$SCR2b = \frac{k_{-3R}.k_{-4R}}{k_{4R} + k_{-3R}}$$

$$SCS2f = \frac{k_{3S}.k_{4S}}{k_{4S} + k_{-3S}}$$

$$SCS2b = \frac{k_{-3S}.k_{-4S}}{k_{4S} + k_{-3S}}$$

Substituting all these SC's leads to the expression:

$$\frac{1}{E_0}\frac{dPR}{dt} =$$

$$= \frac{\begin{array}{c} S.B^2.SCRf.SCR2f.SCS2f + S.B.Q.SCRf.SCR2f.SCSb \\ -PR.B.Q.SCS2f.SCRb.SCR2b - PR.Q^2.SCRb.SCR2b.SCSb \end{array}}{S.B.(SCRf.SCS2f + SCSf.SCR2f) + S.B^2.SCR2f.SCS2f.\left(SCRf.\left(\frac{1}{k_{2R}} + \frac{1}{k_{4R}}\right) + SCSf.\left(\frac{1}{k_{2S}} + \frac{1}{k_{4S}}\right)\right)}$$

$$+ S.Q.(SCRf.SCSb + SCSf.SCRb) + S.Q^2.SCRb.SCSb.\left(\frac{k_{1R}}{k_{-1R}} + \frac{k_{1S}}{k_{-1S}}\right)$$

$$+ S.B.Q.\left(SCR2f.SCSb.\left(SCRf.\left(\frac{1}{k_{2R}} + \frac{1}{k_{4R}}\right) + \frac{k_{1S}}{k_{-1S}}\right) + SCS2f.SCRb.\left(SCSf.\left(\frac{1}{k_{2S}} + \frac{1}{k_{4S}}\right) + \frac{k_{1R}}{k_{-1R}}\right)\right)$$

$$+ B^2.SCR2f.SCS2f + Q^2.SCRb.SCSb + B.Q.(SCR2f.SCSb + SCS2f.SCRb) + PR.B.SCS2f.SCR2b$$

$$+ PS.B.SCR2f.SCS2b + \frac{PR.B^2.SCR2f.SCS2f.k_{-4R}}{k_{4R}} + \frac{PS.B^2.SCR2f.SCS2f.k_{-4S}}{k_{4S}}$$

$$+ PR.Q.SCR2b.SCSb + PS.Q.SCS2b.SCRb + PR.Q^2.SCSb.SCRb.SCR2b.\left(\frac{1}{k_{-1R}} + \frac{1}{k_{-3R}}\right)$$

$$+ PS.Q^2.SCSb.SCRb.SCS2b.\left(\frac{1}{k_{-1S}} + \frac{1}{k_{-3S}}\right)$$

$$+ PR.B.Q.\left(\frac{SCSb.SCR2f.k_{-4R}}{k_{4R}} + SCRb.SCR2b.SCS2f.\left(\frac{1}{k_{-1R}} + \frac{1}{k_{-3R}}\right)\right)$$

$$+ PS.B.Q.\left(\frac{SCRb.SCS2f.k_{-4S}}{k_{4S}} + SCSb.SCS2b.SCR2f.\left(\frac{1}{k_{-1S}} + \frac{1}{k_{-3S}}\right)\right)$$

Several rate constants no longer appear individually in this expression: k_{3R}, k_{3S}, k_{-1R}, k_{-1S}. Others appear only in fixed combinations: k_{1R} and k_{1S} only in the ratios k_{1R}/k_{-1R} and k_{1S}/k_{-1S}; k_{-4R} and k_{-4S} only in the ratios k_{-4R}/k_{4R} and k_{-4S}/k_{4S}; k_{-3R} and k_{-3S} only in the groupings $(1/k_{-1R} + 1/k_{-3R})$ and $(1/k_{-1S} + 1/k_{-3S})$; k_{2R} and k_{2S} only in the groupings $(1/k_{2R} + 1/k_{4R})$ and $(1/k_{2S} + 1/k_{4S})$.

For this kinetic model, the parameter values that might be known differ depending on the type of reaction involved. The most common would be either hydrolysis or synthesis of prochiral diacid esters (in the latter case from the free acid by direct reversal of hydrolysis).

In the case of hydrolysis, an initial rate can be estimated under conditions where Q, PR and PS are all equal to zero. B, which here is H_2O, can be taken as being in constant large excess. This leads to an initial rate of

$$\frac{dPR}{dt} = \frac{E_0.S.SCRf}{1 + S.\left(SCRf.\left(\frac{1}{k_{2R}} + \frac{1}{k_{4R}}\right) + SCSf.\left(\frac{1}{k_{2S}} + \frac{1}{k_{4S}}\right) + \frac{SCRf.}{B.SCR2f} + \frac{SCSf}{B.SCS2f}\right)}$$

Once again the denominator is unchanged on exchange of R and S, so the ratio of initial rates of formation of the enantiomers is SCRf/SCSf, defined again as E. This expression also shows that the apparent K_M value for S would be found as:

$$K_M = \frac{1}{SCRf.\left(\frac{1}{k_{2R}} + \frac{1}{k_{4R}}\right) + SCSf.\left(\frac{1}{k_{2S}} + \frac{1}{k_{4S}}\right) + \frac{SCRf.}{B.SCR2f} + \frac{SCSf}{B.SCS2f}}$$

This will be an apparent K_M value, dependent on the concentration of B (H_2O), although this will normally be in constant large excess.

But in the case of ester synthesis, even under initial rate conditions, Q (H_2O) will not generally be zero, just PR and PS. Hence:

$$\frac{dPR}{dt} = \frac{E_0.S.SCRf.\left(B + \frac{Q.SCSb}{SCS2f}\right)}{\begin{array}{c} S.\left(\frac{SCRf}{SCR2f} + \frac{SCSf}{SCS2f}\right) + S.B.\left(SCRf.\left(\frac{1}{k_{2R}} + \frac{1}{k_{4R}}\right) + SCSf.\left(\frac{1}{k_{2S}} + \frac{1}{k_{4S}}\right)\right) \\ + \frac{S.Q}{B}.\left(\frac{SCRf.SCSb + SCSf.SCRb}{SCR2f.SCS2f}\right) + \frac{S.Q^2}{B}.\frac{SCRb.SCSb}{SCR2f.SCS2f}.\left(\frac{k_{1R}}{k_{-1R}} + \frac{k_{1S}}{k_{-1S}}\right) \\ + S.Q.\left(\frac{SCSb}{SCS2f}.\left[SCRf.\left(\frac{1}{k_{2R}} + \frac{1}{k_{4R}}\right) + \frac{k_{1S}}{k_{-1S}}\right] + \frac{SCRb}{SCR2f}.\left[SCSf.\left(\frac{1}{k_{2S}} + \frac{1}{k_{4S}}\right) + \frac{k_{1R}}{k_{-1R}}\right]\right) \\ + B + \frac{Q^2}{B}.\frac{SCRb.SCSb}{SCR2f.SCS2f} + Q.\left(\frac{SCSb}{SCS2f} + \frac{SCRb}{SCR2f}\right) \end{array}}$$

This expression indicates that the effect of concentration B on the rate will not follow a simple hyperbolic (Michaelis-Menten) relationship. At fixed concentration of B and Q, it would be possible to estimate an apparent K_M for S, but this would be a function of both these concentrations (of B and Q). So it was judged that any information on apparent K_M would probably not be useful in analysing the behaviour.

Again the denominator stays exactly the same on exchanging R and S, so we derive for the ratio of initial rates

$$\frac{Initial\ rate\ R}{Initial\ rate\ S} = \frac{SCRf.\left(B + \frac{Q.SCSb}{SCS2f}\right)}{SCSf.\left(B + \frac{Q.SCRb}{SCR2f}\right)} = \frac{SCRf.\left(\frac{B}{Q} + \frac{SCSb}{SCS2f}\right)}{SCSf.\left(\frac{B}{Q} + \frac{SCRb}{SCR2f}\right)}$$

If B/Q is large enough, then this ratio of rates is close to SCRf/SCSf (i.e. E) as in the previous cases. However, the molar concentration of Q (H_2O) will often be considerably more than that of B (alcohol). The ratios SCSb/SCS2f and SCRb/SCR2f will depend on enzyme characteristics, and may be small, making the limiting case more likely. But if they are significant compared with B/Q, then the initial ratio of enantiomer formation may be noticeably different from E. Interestingly, depending on the enzyme characteristics, the ratio of enantiomers might be higher or lower than E.

As a result of these differences, the choices of input parameters are slightly different for the hydrolysis and esterification cases. For hydrolysis, we again use the initial enantiomer ratio E (= SCRf/SCSf), the apparent K_M value (for S, at fixed B concentration), and the overall equilibrium constant K_{eq}. (For consistency with the esterification case, K_{eq} is here taken as the dimensionless value with its definition including the concentration of H_2O, so its value might be quite small.) Then choose k_{2R} as the rate constant assigned a value to set the timescale.

Now define a first set of input parameters based on ratios of rate constants: k_{4R}/k_{2R}, $k_{2R}/(E.k_{2S})$ and k_{4S}/k_{2S}. Each of these is dimensionless, and expected to be of the order of magnitude of 1. The overall rate expression also includes groups where k_{2R} and k_{4R}, and k_{2S} and k_{4S}, appear together.

To make use of the expression for the apparent K_M, we need relationships for SCR2f and SCS2f. Define E2 (=SCR2f/SCS2f), but actually use E2/E as the dimensionless input parameter. It will often be of the order of 1, but possibly far away if the enantiopreference of the enzyme is very different in

the acylation and de-acylation phases of the reaction. Also define as a parameter the dimensionless ratio SCR2f/SCRf. This could also be of the order of 1, but could be considerably smaller and still give a sensible reaction progress, because B (H_2O) >> S. With these two parameters set, SCRf can be calculated from the K_M value.

Now introduce SCRf/b (=SCRf/SCRb), which again will be found empirically to be very important in determining the behaviour. Also define the parameter SCSf/b (=SCSf/SCSb). Both these parameters will have some tendency to increase with K_{eq}, but the relationship will not be simple, because the favourability may differ substantially for the two part reactions of forming and then breaking down the E* intermediate.

Now use the two equilibrium relationships to find all remaining SC's. Racemisation here requires all 4 reaction steps for each enantiomer:

$$K_{rac} = 1 = \frac{k_{1R}.k_{2R}.k_{3R}.k_{4R}.k_{-1S}.k_{-2S}.k_{-3S}.k_{-4S}}{k_{1S}.k_{2S}.k_{3S}.k_{4S}.k_{-1R}.k_{-2R}.k_{-3R}.k_{-4R}} = \frac{SCRf.SCR2f.SCSb.SCS2b}{SCSf.SCS2f.SCRb.SCR2b}$$

While the overall reaction equilibrium constant

$$K_{eq} = \frac{k_{1R}.k_{2R}.k_{3R}.k_{4R}}{k_{-1R}.k_{-2R}.k_{-3R}.k_{-4R}} = \frac{SCRf.SCR2f}{SCRb.SCR2b}$$

We now need 4 more parameters, one for each part reaction and enantiomer. From the overall rate expression the ratios k_{1R}/k_{-1R}, k_{1S}/k_{-1S}, k_{4R}/k_{-4R}, k_{4S}/k_{-4S} look attractive, but they all have dimensions because one of the rate constants is second order. So it is preferred to use dimensionless ratios like k_{-1R}/k_{2R}, which can be seen as controlling the partitioning of the intermediate ESR. Hence we use the following ratios as input parameters: k_{-1R}/k_{2R}, k_{-1S}/k_{2S}, k_{-3R}/k_{4R} and k_{-3S}/k_{4S}. For each part reaction and enantiomer we now have 1 rate constant, two SC's and this final parameter, allowing all rate constants to be calculated.

In the case of an esterification reaction, we again use the overall equilibrium constant K_{eq} as one input parameter, along with k_{2R} as the rate constant that sets the timescale. The ratio E (= SCRf/SCSf) is also retained, even though in this case it may not give the initial ratio of enantiomer formation. The true dissociation constant for the E.S_R complex ($K_S = k_{-1R}/k_{1R}$) was chosen as in input instead of any apparent K_M value. It is unlikely to be known accurately, but it will often be possible to make a reasonable order of magnitude estimate, perhaps making use of kinetic parameters. This ratio of rate constants also appears in the full rate expression.

Now select the parameter k_{-1R}/k_{2R} as in the hydrolysis case, and SCRf/b (=SCRf/SCRb), again found empirically to be an influential parameter. Combining with k_{2R} and K_S then allows calculation of k_{1R}, k_{-1R}, k_{-2R} and both SCRf and SCRb. Then using E we get SCSf.

To proceed, define 3 more parameters as used in the hydrolysis case and discussed there: E2/E, SCR2f/SCRf, and SCSf/b (=SCSf/SCSb). With values of SCRf, SCRb and SCSf, together with the relationships to K_{rac} and K_{eq} as in the hydrolysis case, we can now calculate all 8 SC's.

For the first part reaction with the S enantiomer, we have SCSf and SCSb. Using two more parameters, $k_{2R}/(E.k_{2S})$ and k_{-1S}/k_{2S}, as for the hydrolysis case (again discussed there), allows

calculation of all 4 rate constants. For the second part reaction with the R enantiomer, choose another two parameters as used and discussed for hydrolysis: k_{4R}/k_{2R} and k_{-3R}/k_{4R}. Combined with SCR2f and SCR2b, all rate constants are determined. For the S enantiomer, we use k_{-3S}/k_{4S} as for hydrolysis, but it seems more consistent to select $k_{4R}/(E.k_{4S})$ as the final parameter (not k_{4S}/k_{2S}).

Estimation of ee at 95% of equilibrium conversion

This was made by interpolation of the table of conversion and product ee output from the calculated progress simulation. The K_{eq} value is defined for the reaction to produce the R enantiomer (arbitrarily taken as being the predominant one). Hence the "equilibrium" conversion is calculated as that which would be achieved if the enzyme were completely enantiospecific. To the extent that S product is also formed, the observed conversion can exceed this "equilibrium" value. (At final true equilibrium there would be equal concentrations of the two enantiomers, but of course this equilibrium should not be approached in a preparative reaction.) Formation of the unfavoured enantiomer will make reaching 95% of equilibrium conversion rather easier – if formation of 95% of the equilibrium concentration of the favoured enantiomer were required, the ee value found would be even lower.