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ORIGINAL PAPER

A new mathematical model for the enzymatic kinetic resolution of racemates

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Abstract A mathematical model is presented for the kinetic resolution of racemates. It takes all intermediate binding steps into account and assumes that such steps are reversible. The model describing dynamics of the chiral reaction products consists of two nonlinear differential equations. With this model, the enantioselectivity of enzyme has been studied. Mathematical and numerical simulation of the model show that there are several ways to control the enantiomeric ratio (E) but the affinity and the binding rates of the intermediate enzyme complex to the racemic substrates are the key steps for the enzyme enantioselectivity.

Keywords Kinetic resolution of racemate · Transesterification · Enantioselectivity · Lipase · Mathematical modeling · Organic solvent

1 Introduction

Homochirality is one of the most exciting properties in the molecular components of living organisms. It is known that L-forms of amino acids serve as building blocks of proteins, and D-sugars are incorporated in the backbone of DNA, which are responsible for the synthesis of chiral biomolecules such as proteins and enzymes [1–3]. The biological receptor systems also recognize compounds with a specific chirality. Two

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enantiomers of a chiral molecule can exhibit completely different biological activities. For example, one enantiomer of a racemic mixture can be an active drug whereas the other can exhibit fatal toxicity within the body [4]. Therefore, the separation of racemates and enhancement of one of the enantiomers are extremely important for the pharmaceutical and agricultural purposes [5,6]. There are several analytical methods used for the enantio-separation such as diastereomeric crystallization [7], chromatographic techniques [8], asymmetric catalysis [9,10] and biocatalysis [11]. Only the biocatalytic kinetic resolution of racemic mixtures is considered in this study.

Commercial implementation of enzyme-catalyzed processes requires derivation of the rate expression and estimation of the kinetic parameters, which are two important steps in the process development. Rate equations and parameter values describe the performance of the reactor in terms of the major factors that control the individual reaction (e.g., temperature, enzyme loading, concentrations of substrates, etc.) [12,13].

Kinetic of racemates have been studied extensively. Most of these studies have focused on model systems, which often lump several reaction steps into a single step and contain only a few different chemical species and/or enzyme complexes. Berendsen et al. [12] studied a detailed model developed based on reversible ping-pong mechanism by taking both reversibility and competitive inhibition into account. Xiong et al. [14] studied the kinetic resolution of one enantiomer, but not the resolution of both enantiomers simultaneously.

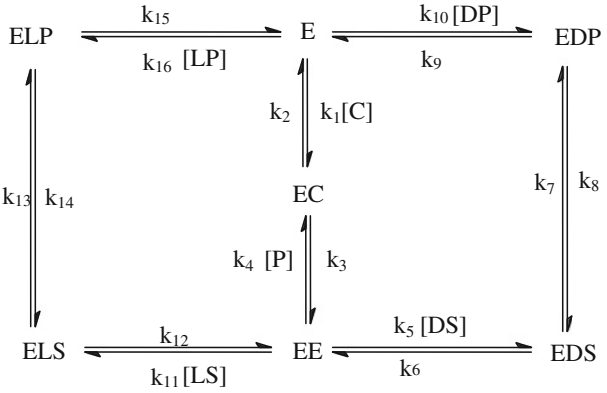
To study enantioselective biocatalysis and determine impacts of individual reaction steps on each chiral product, we developed a general mechanistic model. The model considers all individual reaction steps as fully reversible and takes both enantiomers as competing substrates in binding an enzyme.

2 Development of the model for an enantioselective reaction

The kinetic resolution of racemates is a popular method to synthesis enantiomerically pure compounds. The kinetic mechanism of an enantioselective reaction follows ping-pong bi–bi mechanism [15,16]. Taking the competition of both substrate enantiomers for the enzyme active site into account, a kinetic model based on reversible ping-pong bi–bi mechanism was applied to describe the kinetic behavior of such reactions, e.g., lipase catalyzed transesterification.

In development of this model, we assumed the following assumptions hold [17–19]: (1) even sized enzymes are uniformly distributed and dissolved in the solution medium, (2) enzyme deactivation is negligible, (3) concentrations of all intermediate enzyme complexes are at equilibrium during the measurement, (4) inhibition of products was negligible, (5) mass transfer resistance is negligible, and finally (5) all other reaction conditions are constant during measurement.

The mechanism proposed for enantioselective reactions is depicted in Scheme 1. In this mechanism, the enzyme E first reacts with the excess component C and the by-product P and the enzyme–acyl complex EC are produced. In trans/esterification reactions the excess component is usually the acyl donor component. Then, the enzyme complex EC surrogates to the enzyme complex EE by a relaxation reaction. Then both of the racemates LS and DS compete to bind the active site of the enzyme complex EE.



Scheme 1 A mechanism for racemic reactions for enantioselective reactions

Consequently, two parallel pathways exist for the decomposition of the acyl–enzyme intermediate. When DS combines to EE, the right side of the loop on this mechanism is functional, and when LS binds to EE then the left loop of the mechanism becomes functional (see Scheme 1). Depending on the substrate bound to the enzyme EE, either EDS or ELS complexes are formed and they soon get into the relaxation reactions to produce EDP and ELP complexes, respectively. In the final step, the enzyme releases from the end products DP and LP. Then it becomes free in the reaction medium to react with next excess component [22].

This is a general mechanism and it is assumed that all the steps are reversible. The advantage of this mechanism is that it can later be adapted to any type of enantioselective reaction whose intermediates will be determined by their own chemical mechanisms by equating the appropriate reversible rate constant to zero.

Derivation of rate equations for complex enzymatic reactions is a tedious task which requires manipulation with massive algebraic expressions. A number of derivation methods were proposed for complex enzymatic systems [20,21]. We use the algebraic method to derive rate equations for this mechanism here. There are 11 dynamic variables in this system: there are two substrates (LS and DS) and two products (LP and DP). There is also a substrate C we assume it is in abundance and its product P which will be considered as parameters in our derivation. Moreover, there is a free enzyme form (E) and six enzyme-substrate complexes. We assume that total concentrations of enzyme and its substrates LS and DS are constant over the course of the reaction. Therefore, we have

$$[E]_0 = [E] + [EC] + [EE] + [EDS] + [ELS] + [EDP] + [ELP] \tag{2.1}$$

$$[DS]_0 = [DS] + [EDS] + [EDP] + [DP] \tag{2.2}$$

$$[LS]_0 = [LS] + [ELS] + [ELP] + [LP] \tag{2.3}$$

Here $[E]_0\,[LS]_0$ and $[DS]_0$ are the initial concentration of the enzyme and its two substrates LS and DS, respectively. These assumptions leaves only 8 free dynamic variables, which are EC, EE, EDS, ELS, EDP, ELP, DP and LP. According to mass

action kinetics, the following eight differential equations describe how concentrations of each variable changes over time:

$$\frac{d[EC]}{dt} = k_1[E][C] - k_2[EC] - k_3[EC] + k_4[EE][P] \tag{2.4}$$

$$\frac{d[EE]}{dt} = k_3[EC] - k_4[EE][P] - k_5[EE][DS] + k_6[EDS] - k_{11}[EE][LS] + k_{12}[ELS] \tag{2.5}$$

$$\frac{d[EDS]}{dt} = k_5[EE][DS] - k_6[EDS] - k_7[EDS] + k_8[EDP] \tag{2.6}$$

$$\frac{d[EDP]}{dt} = k_7[EDS] - k_8[EDP] - k_9[EDP] + k_{10}[E][DP] \tag{2.7}$$

$$\frac{d[ELS]}{dt} = k_{11}[EE][LS] - k_{12}[ELS] - k_{13}[ELS] + k_{14}[ELP] \tag{2.8}$$

$$\frac{d[ELP]}{dt} = k_{13}[ELS] - k_{14}[ELP] - k_{15}[ELP] + k_{16}[E][LP] \tag{2.9}$$

And the equations for rates of change of DP and LP concentrations are

$$\frac{d[DP]}{dt} = k_9[EDP] - k_{10}[E][DP] \tag{2.10}$$

$$\frac{d[LP]}{dt} = k_{15}[ELP] - k_{16}[E][LP] \tag{2.11}$$

Under the quasi steady state assumption on the enzyme complexes, the differential equations governing dynamics of the products become,

$$\frac{d[DP]}{dt} = \frac{V_{\max 1}[DS]\,(\Theta_1+\Theta_2[DP]+\Theta_3[LP]) - V_{\max 2}[DP]\,(\Theta_4+\Theta_5[DS]+\Theta_6[LS])}{(\Omega_1+\Omega_2[LP]+\Omega_3[DP])\,(\Theta_4+\Theta_5[DS]+\Theta_6[LS]) + (\Omega_4[DS]+\Omega_5+\Omega_6[LS])\,(\Theta_1+\Theta_2[DP]+\Theta_3[LP])} \tag{2.12}$$

$$\frac{d[LP]}{dt} = \frac{V_{\max 3}[LS]\,(\Theta_1+\Theta_2[DP]+\Theta_3[LP]) - V_{\max 4}[LP]\,(\Theta_4+\Theta_5[DS]+\Theta_6[LS])}{(\Omega_1+\Omega_2[LP]+\Omega_3[DP])\,(\Theta_4+\Theta_5[DS]+\Theta_6[LS]) + (\Omega_4[DS]+\Omega_5+\Omega_6[LS])\,(\Theta_1+\Theta_2[DP]+\Theta_3[LP])} \tag{2.13}$$

where, Θ_i's, Ω_i,'s and $V_{\max j}$'s are as in Eqs. (5.19), (5.27) and (5.29)–(5.31), respectively. Details of this derivation are given in the appendix. In an enzymatic reaction, initial enzyme concentration is always much smaller than the initial substrate concentrations, which simplifies Eqs. (2.2) and (2.3) to

$$[DS]_0 = [DS] + [DP] \tag{2.14}$$

$$[LS]_0 = [LS] + [LP] \tag{2.15}$$

Notice that, in Eq. (2.12), if at least one of k_5, k_7 and k_9 is zero then $V_{max\,1}$ becomes zero and DP is not produced. Same is true for LP if at least one of k_{11}, k_{13} and k_{15} is zero [see Eq. (2.13)].

Without loss of generality, we assume $[LS]_0 = [DS]_0 = 1$ hereafter and do our mathematical analysis and simulations under this assumption, which can be achieved by dividing both sides of Eqs. (2.14) and (2.15) by $[DS]_0$ and $[LS]_0$, respectively and defining new variables as $[DP]/[DS]_0$ and $[LP]/[LS]_0$.

3 Results

3.1 Steady state analysis of the model

Suppose that the system described by Eqs. (2.12) and (2.13) has a steady state at $([DP^*], [LP^*])$. There are no temporal changes in the concentrations of DP and LP at a steady state, hence $\frac{d[DP]}{dt}\big|_{([DP^*],[LP^*])} = \frac{d[LP]}{dt}\big|_{([DP^*],[LP^*])} = 0$ has to hold. Therefore, the steady state of this system is described by the following two algebraic equations

$$\frac{V_{max\,1}[DS^*]\left(\Theta_1+\Theta_2[DP^*]+\Theta_3[LP^*]\right)-V_{max\,2}[DP^*]\left(\Theta_4+\Theta_5[DS^*]+\Theta_6[LS^*]\right)}{\left\{\left(\Omega_1+\Omega_2[LP^*]+\Omega_3[DP^*]\right)\left(\Theta_4+\Theta_5[DS^*]+\Theta_6[LS^*]\right)+\ldots\left(\Omega_4[DS^*]+\Omega_5+\Omega_6[LS]^*\right)\left(\Theta_1+\Theta_2[DP^*]+\Theta_3[LP^*]\right)\right\}} = 0 \tag{3.1}$$

$$\frac{V_{max\,3}[LS^*]\left(\Theta_1+\Theta_2[DP^*]+\Theta_3[LP^*]\right)-V_{max\,4}[LP^*]\left(\Theta_4+\Theta_5[DS^*]+\Theta_6[LS^*]\right)}{\left\{\left(\Omega_1+\Omega_2[LP^*]+\Omega_3[DP^*]\right)\left(\Theta_4+\Theta_5[DS^*]+\Theta_6[LS^*]\right)+\ldots\left(\Omega_4[DS^*]+\Omega_5+\Omega_6[LS]^*\right)\left(\Theta_1+\Theta_2[DP^*]+\Theta_3[LP^*]\right)\right\}} = 0 \tag{3.2}$$

Since the terms in the denominators of these two equations are always nonnegative, they simplify to,

$$V_{max\,1}[DS^*]\left(\Theta_1+\Theta_2[DP^*]+\Theta_3[LP^*]\right) - V_{max\,2}[DP^*]\left(\Theta_4+\Theta_5[DS^*]+\Theta_6[LS^*]\right) = 0 \tag{3.3}$$

$$V_{max\,3}[LS^*]\left(\Theta_1+\Theta_2[DP^*]+\Theta_3[LP^*]\right) - V_{max\,4}[LP^*]\left(\Theta_4+\Theta_5[DS^*]+\Theta_6[LS^*]\right) = 0 \tag{3.4}$$

If any of the matching steps in both loops are irreversible then this system has a steady state at $([DS^*], [LS^*]) = (0, 0)$, which is equivalent to $([DP^*], [LP^*]) = (1, 1)$ from Eqs. (2.14)–(2.15). To see this let's assume both $V_{max\,2} = V_{max\,4} = 0$, since $\Theta_1 + \Theta_2[DP^*] + \Theta_3[LP^*] > 0$ the solution of Eqs. (3.3)–(3.4) becomes $([DS^*], [LS^*]) = (0, 0)$. $V_{max\,2}$ can be zero when one or more of the rate constants k_6, k_8 and k_{10} are zero, and $V_{max\,4}$ vanishes when one or more of the rate constants k_{12}, k_{14} and k_{16} are zero. On the other hand, when one of k_2 and k_{4P} or both are zero, Θ_4 becomes zero [see Eq. (5.19)]. The steady state equations given in Eqs. (3.3)–(3.4) simplifies to:

$$V_{\max 1}[DS^*]\left(\Theta_1+\Theta_2[DP^*]+\Theta_3[LP^*]\right) -V_{\max 2}[DP^*]\left(\Theta_5[DS^*]+\Theta_6[LS^*]\right)=0 \quad (3.5)$$

$$V_{\max 3}[LS^*]\left(\Theta_1+\Theta_2[DP^*]+\Theta_3[LP^*]\right) -V_{\max 4}[LP^*]\left(\Theta_5[DS^*]+\Theta_6[LS^*]\right)=0 \quad (3.6)$$

From Eqs. (3.5)–(3.6), it is straightforward that $[DS^*]=[LS^*]=0$ is a steady state. Therefore, $[DP^*]=[LP^*]=1$ is again a steady state.

When all the steps in this system are reversible, it has a steady state at $([DP^*],[LP^*])$ such that $[DP^*]<1$ and $[LP^*]<1$.

3.2 Dynamics analysis of the model

The mechanism depicted in Scheme 1 has a symmetrical structure. We look at how the dynamics of the final products LP and DP can be controlled when the macthing steps in the left and right loops are irreversible.

Case A When the parameters k_6 and k_{12} are both zero, the reverse reactions $ELS \overset{k_{12}}{\rightarrow} LS+EE$ and $EDS \overset{k_6}{\rightarrow} DS+EE$ do not occur, which results in $V_{\max 2}=V_{\max 4}=0$ and simplifies $V_{\max 1}$ and $V_{\max 3}$ to

$$V_{\max 1}=\frac{k_5k_7k_9[E]_{tot}}{k_7k_9}=k_5[E]_0 \quad \text{and} \quad V_{\max 3}=\frac{k_{11}k_{13}k_{15}[E]_{tot}}{k_{13}k_{15}}=k_{11}[E]_0 \quad (3.7)$$

Since all the terms in Eqs. (2.12) and (2.13) are common except $V_{\max 1}$ and $V_{\max 3}$, the only way to dynamically control the production of LP and DP is to change ratio of k_5 and k_{11}, which is defined as the ratio of enantioselectivity [17]. In Fig. 1, the temporal changes in LP and DP concentrations are simulated numerically. To produce this simulation, we numerically solved the model equations in Eqs. (2.12)–(2.13) starting from the initial values $[LP](0)=0$ and $[DP](0)=0$ until it reaches the steady state. We first fixed the parameter values. In this particular simulation, we set $k_6=k_{12}=0$, $k_{11}=10$, $[E]_0=0.01$ and all the other parameters to 1. As seen in this simulation, all DS ends up becoming DP and all LS is converted into LP but the production of LP is faster than the production of DP due to higher affinity of the EE form of the enzyme against LS ($k_{11}=10$) compared to its affinity to DS ($k_5=1$). For this parameter settings, all LS becomes LP and only about 20 % of DS becomes DP until t = 1,000. The remaining 80 % of DS is converted into DP between t = 1,000 and t = 6,000. After t = 6,000, [DP] and [LP] reach their steady states values $[DP^*]=[LP^*]=1$. The concentrations are dimensionless and time is in arbitrary unit in these simulations.

Case B When $k_8=k_{14}=0$, the reverse reactions $ELP \overset{k_{14}}{\rightarrow} ELS$ and $EDP \overset{k_8}{\rightarrow} EDS$ do not occur, which results in again $V_{\max 2}=V_{\max 4}=0$. In this particular case, $V_{\max 1}$ and $V_{\max 3}$ become equal to

$$V_{\max 1}=\frac{k_5k_7}{k_6+k_7}[E]_0 \quad \text{and} \quad V_{\max 3}=\frac{k_{11}k_{13}}{k_{12}+k_{13}}[E]_0 \quad (3.8)$$

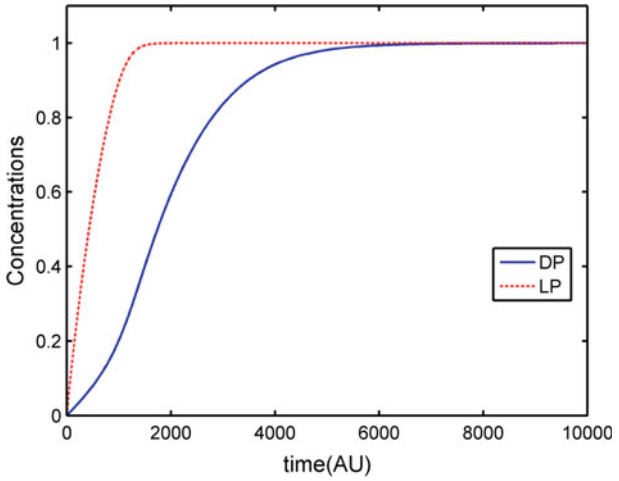


Fig. 1 Simulation of LP and DP concentrations over time when $k_{11} = 10$, $k_6 = k_{12} = 0$ and $[E]_0 = 0.01$. In this simulation the initial conditions are $[LP](0) = 0$ and $[DP](0) = 0$ and all other parameter values are set to be 1. As seen in this figure, the production of LP is faster than the production of DP since the enzyme's affinity to LS is 10 times larger than its affinity to DS

All the terms in Eqs. (2.12)–(2.13) are common but the maximum rates $V_{\max 1}$ and $V_{\max 3}$. The dynamics of LP and DP productions can be controlled by the parameters k_5, k_7, k_6, k_{11}, k_{13} and k_{12}. When $k_7/(k_6 + k_7) = k_{13}/(k_{12} + k_{13})$, the only way to control time courses for the production of LP and DP are to change the ratio of k_5 and k_{11} (enanioslectivity parameters). In a special case, if $k_6 \ll k_7$ and $k_{12} \ll k_{13}$ then $k_7/(k_6 + k_7)$ and $k_{13}/(k_{12} + k_{13})$ become roughly same and approximately equal to 1, which means that the rate of conversion of EDS into EE and DS is significantly slower than the rate of conversion of EDS into EDP, and the rate of conversion of ELS into EE and LS is much slower than the rate of conversion of ELS into ELP. When $k_7/(k_6 + k_7) \neq k_{13}/(k_{12} + k_{13})$ and $k_5 = k_{11}$, the ratio of $k_7/(k_6 + k_7)$ and $k_{13}/(k_{12} + k_{13})$ determines the dynamics of DP and LP productions. As an example, we numerically solved the model equations with an initial condition $[LP](0) = 0$ and $[DP](0) = 0$ when $k_8 = k_{14} = 0$, $k_{13} = 10$, $[E]_0 = 0.01$ and the values of all the other parameters are 1. The result is shown in Fig. 2. As shown in this simulation, both LP and DP concentrations reach the same steady state $[DP^*] = [LP^*] = 1$ but the dynamics of [LP] is faster than the dynamics of [DP] due to the faster conversion rate of ELS into ELP ($k_{13} = 10$). However, the difference in DP and LP concentrations during transient period is significantly smaller than the difference we observed in Fig. 1. For this parameter values, the modified form of the enzyme EE binds to LS and DS equally fast and starts to convert DS and LS into DP and LP, respectively. Since $k_{13} = 10$ and $k_7 = 1$, unlike Case A, the dynamics of LP is slightly faster than the dynamics of DP and the enzyme works on both of the substrates at comparable rates. In case A, the enzyme prefers LS over DS until all LS becomes LP.

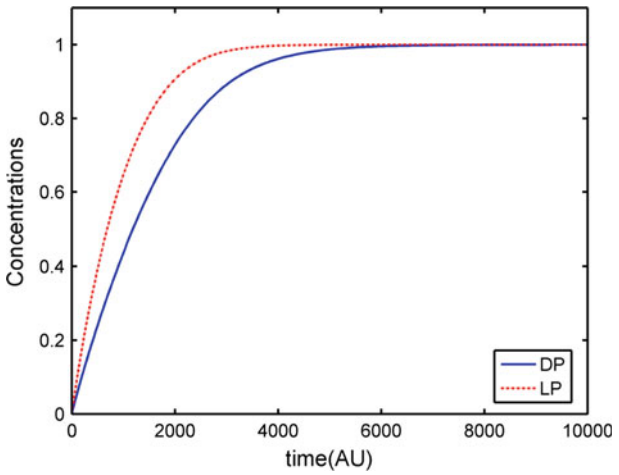


Fig. 2 Changes in LP and DP concentrations over time when $k_8 = k_{14} = 0$ and $[E]_0 = 0.01$. In this run, all other parameters are set to be equal to 1 except k_{13}. This parameter is chosen to be $k_{13} = 10$, which 10 times bigger than k_7. As seen, the production of LP occurs faster than that of DP but both LP and DP concentrations approach to 1 over time

Figure 2 shows LP and DP concentrations over time starting from $[LP](0) = 0$ and $[DP](0) = 0$. The parameter values for this simulation are $k_8 = k_{14} = 0$ and all other parameters are set to be equal to 1 except k_{13}, which is chosen to be $k_{13} = 10$ to make the conversion of ELS into ELP 10 times faster than the conversion of EDS into EDP. As seen in this simulation, the production of LP occurs faster than that of DP but both LP and DP concentrations converge to 1.

Case C When $k_{10} = k_{16} = 0$, the maximum rates $V_{\max 2}$ and $V_{\max 4}$ become zero. This corresponds to the system in which the reverse reactions $ELP \overset{k_{16}}{\rightarrow} E + LP$ and $EDP \overset{k_{10}}{\rightarrow} E + DP$ do not occur. $V_{\max 1}$ and $V_{\max 3}$ simplifies to

$$V_{\max 1} = \frac{k_5 k_7 k_9}{k_6(k_8 + k_9) + k_7 k_9}[E]_0 \quad \text{and} \quad V_{\max 3} = \frac{k_{11} k_{13} k_{15}}{k_{12}(k_{14} + k_{15}) + k_{13} k_{15}}[E]_0$$

The dynamics of LP and DP can be controlled by changing the parameters k_5, k_6, k_7, k_8, k_9, k_{11}, k_{12}, k_{13}, k_{14} and k_{15} since all the other terms in the model equations are shared but $V_{\max 1}$ and $V_{\max 3}$. When $\frac{k_7 k_9}{k_6(k_8+k_9)+k_7 k_9} = \frac{k_{13} k_{15}}{k_{12}(k_{14}+k_{15})+k_{13} k_{15}}$ is true then the only way to control the production of LP and DP are to change the ratio of k_5 and k_{11}.

Notice that for large values of k_9 and k_{15}, $V_{\max 1} \approx \frac{k_5 k_7}{k_6 + k_7}[E]_0$ and $V_{\max 3} \approx \frac{k_{11} k_{13}}{k_{12} + k_{13}}[E]_0$ hold and this case simplifies to Case B above. Moreover, for large values of k_7 and k_{13}, $V_{\max 1} \approx k_5[E]_0$ and $V_{\max 3} \approx k_{11}[E]_0$, and the production of the final products LP and DP are determined by only k_5 and k_{11} (see Fig. 3).

When all the steps in the reaction are reversible, there must be some DS and LS at the steady state giving the steady state values for both DP and LP <1. How much of

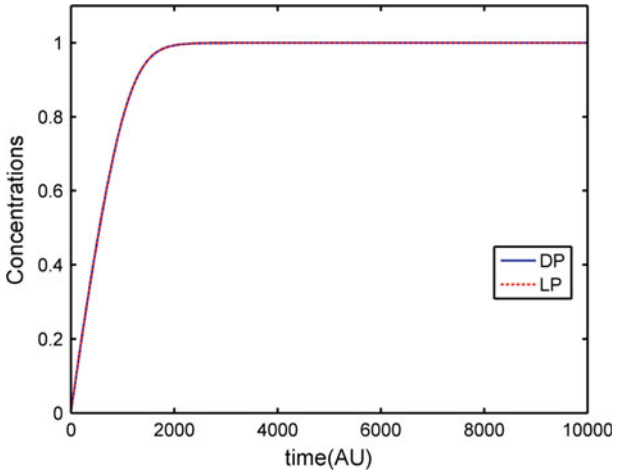


Fig. 3 Simulation of LP and DP concentrations over time. In this simulation, we took $k_{10} = k_{16} = 0$ and $[E]_0 = 0.01$. The values of the parameters are $k_7 = k_{13} = 10^4$, $k_{14} = 0.1$, $k_{15} = 10$ and all the other parameters are set to be 1. Since k_7 and k_{13} are too big, choosing different values for k_{14} and k_{15} has no effect on the production of LP and DP, the curves for [LP] and [DP] are overlapping

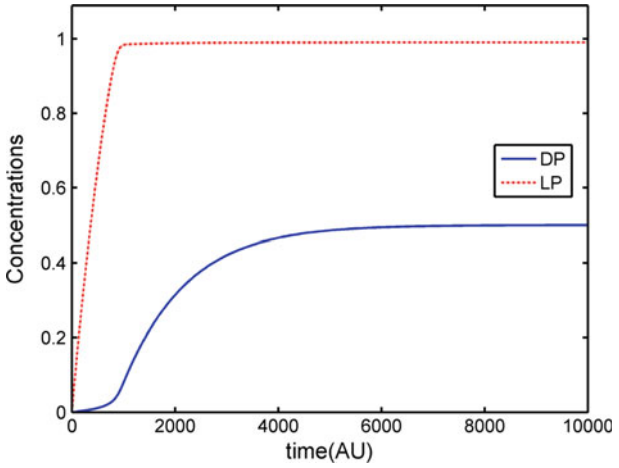


Fig. 4 Simulated LP and DP concentrations over time when all the reaction steps are reversible. In this simulation, we took $[E]_0 = 0.01$ and all the parameter values are set to be 1 except k_{11} which is $k_{11} = 100$. Similar to the previous runs, the initial conditions are $[LP](0) = 0$ and $[DP](0) = 0$. For this parameter settings, the enzyme works on the conversion of LS into LP until t = 1,000. Then it starts working on the conversion of DS into DP

DS and LS are converted into LP and DP at steady state are determined solely by the selection of the values for the parameters.

In Fig. 4, the change in LP and DP concentrations over time are given when all the reaction steps are reversible. For this simulation, all the parameter values are set to be 1 except k_{11} which is the parameter that determines how fast the enzyme binds to the substrate LS. This parameters is chosen as $k_{11} = 100$ and the total enzyme

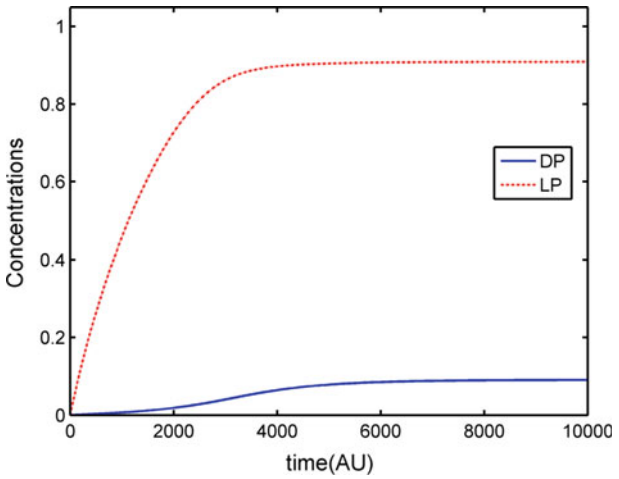


Fig. 5 Simulation of LP and DP concentrations when all the reaction steps are reversible. For this simulation, we chose $k_{11} = 100$ and $k_2 = 10$, and all the other parameter values are set to be 1. The initial conditions are $[LP](0) = 0$ and $[DP](0) = 0$

concentration is $[E]_0 = 0.01$. Similar to the previous runs, the initial conditions are $[LP](0) = 0$ and $[DP](0) = 0$. For this set of parameter values, the steady state values are calculated as [DP*]=0.4998 and [LP*]=0.9901. The enzyme acts on LS much faster than DS and converts LS into LP, and then its starts to work on the conversion DS into DP(when time is about t=1,000). Only small fraction of DS (<1 %) is converted into DP until t=1,000. Due to higher k_{11} value, the steady state of LP is much larger than the steady state of DP.

We also investigated how the reaction steps before formation of EE form of the enzyme affects the dynamics of LP and DP productions. We numerically solved the model equations by setting $k_2 = 10$ when $[E]_0 = 0.01$ (Fig. 5). For this parameter settings, the steady state values are calculated as [DP*]= 0.091 and [LP*]=0.909 which are smaller than the steady state values calculated in Fig. 4. Similar to the pervious simulation, the enzyme acts on LS much faster and converts it into LP. Then it starts working on DS. However, the steady state values for both DP and LP are smaller due to the faster backward rate in the step $E + C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EC$.

We studied how slower production rate at the final step of LP production affects the production of LP and DP when EE form of the enzyme binds to LS faster than DS. For this, we set $k_{11} = 10$ and $k_{15} = 0.02$ and all the other parameters to 1 and run a simulation with an initial condition $[LP](0) = 0$ and $[DP](0) = 0$ (Fig. 6). Although EE binds to LS 10 times faster than DS, it is still possible make production of LP slower by choosing the other forward rate constants small enough. For this parameter settings, the steady states of LP is smaller than the steady state of DP, which is calculated as [DP*]=0.496 and [LP*]=0.165. A similar result can be obtained by choosing k_{13} small enough.

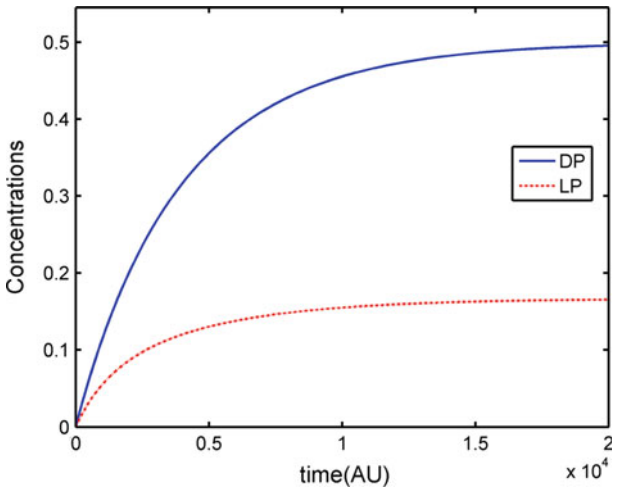


Fig. 6 Simulation of LP and DP concentrations over time when all the reaction steps are reversible. For this simulation, we set $k_{11} = 10$, $k_{15} = 0.02$ and all the other parameter values to 1. The initial conditions are $[LP](0) = 0$ and $[DP](0) = 0$. Since the rate constant at the final step of the production of DP is small enough ($k_{15} = 0.02$), LP is accumulated at a smaller rate than DP despite the fact that the enzyme's binding rate to LS 10 times faster ($k_{11} = 10$) than its binding rate to DS ($k_5 = 1$).

Affects of the enzyme concentration on the dynamics of LP and DP production were investigated by changing the total enzyme concentration in our simulation (Fig. 7). The values of all the parameters are set to be 1 when $k_{11} = 10$. The initial conditions are $[LP](0) = 0$ and $[DP](0) = 0$. The total enzyme concentrations were chosen as 5×10^{-3}, 10^{-2} and 5×10^{-2}. The amount of the enzyme increases the production of rate of LP and DP as expected. However, the steady state concentrations of LP and DP remain unchanged.

4 Discussions

A new mathematical model was proposed for the kinetic resolution of racemic mixtures. The model simplifies to a 2-dimensional nonlinear differential equations. Through mathematical analysis and numerical simulation of the model, enhancement of enantioselectivity in racemic mixtures has been studied.

Our analysis of the model shows that all racemic substrates LS and DS are converted into the chiral products LP and DP when at least one of the intermediate reaction step is irreversible (Figs. 1, 2, 3). On the other hand, they are partially converted into their respective products when all the steps are reversible. Amount of racemic substrates converted into the chiral products are determined by selection of the parameter values.

The reaction steps until the formation of the complex EE have same effect on the dynamics of the products. Our study also shows that the binding rates of EE to either of the racemic substrates LS or DS is the key step for the enantioselectivity.

Enantiomeric ratio is a measure of enantio-enhancement of racemic solutions. Previously, Chen and Sih [23–25] studied enantioselective biocatalysis in organic solvent

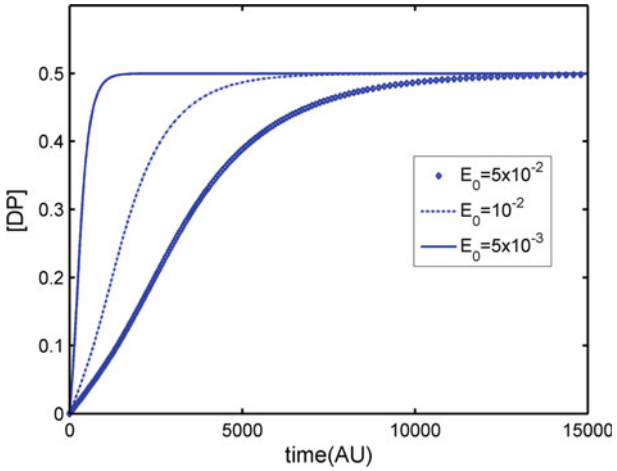


Fig. 7 Dependence of DP production on the enzyme concentration. In this simulation, we numerically solved the model for three different concentrations of the enzyme: 5×10^{-3} (*the diamond*), 10^{-2} (*the dotted curve*) and 5×10^{-2} (*the solid line*). In this run, all the parameters values are set to be 1 when $k_{11} = 10$. Total enzyme concentration does not change the steady state concentration of DP. However, the production of LP occurs at a faster rate for higher concentration of the enzyme

and used overall reaction rates to define enantiomeric ratio as the ratio of forward binding rate constants of the racemic substrates. They neglected the intermediate binding steps. In this study, we have shown that their definition is true when both EDS and ELS formation steps are irreversible (Case A) and their measure for enantiomeric ratio can only be used for irreversible kinetic resolution of racemic reaction mixtures.

We have also mathematically shown that the enantiomeric ratio reduces to some simplified forms involving individual rate constants when the system is not fully reversible (Cases A–C). Under special circumstances, all such ratios simplify to the enantiomeric ratio defined by Chen and Shi in [23–25].

Our model is applicable to any type of kinetic resolution of enzyme catalysed racemic solutions, such as transesterification reactions. It is known that the transesterification reactions contain an important acylation step and acyl–enzyme complex formation can be reversible [26] or irreversible [27]. Such complexes are crucial intermediates in all lipase catalyzed reactions [28]. Thus, the enantioselectivity strongly depends on the affinity of the acylated enzyme complex for transesterification reactions (The complex form of the enzyme EE in the model).

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5 Appendix: Derivation of the model equations

The system of eight differential equations in Eqs. (2.4)–(2.11) and the mass conservation equations in Eqs. (2.1)–(2.3) describe dynamic evolution of the reaction network in Scheme 1. To simplify this model further, we assume that this system is at quasi-

steady state and all the enzyme complexes stay constant over the course of the reaction. Therefore,

$$\frac{d[EC]}{dt} = 0 = k_1[E][C] - k_2[EC] - k_3[EC] + k_4[EE][P] \tag{5.1}$$

$$\frac{d[EE]}{dt} = 0 = k_3[EC] - k_4[EE][P] - k_5[EE][DS] + k_6[EDS] - k_{11}[EE][LS] + k_{12}[ELS] \tag{5.2}$$

$$\frac{d[EDS]}{dt} = 0 = k_5[EE][DS] - k_6[EDS] - k_7[EDS] + k_8[EDP] \tag{5.3}$$

$$\frac{d[EDP]}{dt} = 0 = k_7[EDS] - k_8[EDP] - k_9[EDP] + k_{10}[E][DP] \tag{5.4}$$

$$\frac{d[ELS]}{dt} = 0 = k_{11}[EE][LS] - k_{12}[ELS] - k_{13}[ELS] + k_{14}[ELP] \tag{5.5}$$

$$\frac{d[ELP]}{dt} = 0 = k_{13}[ELS] - k_{14}[ELP] - k_{15}[ELP] + k_{16}[E][LP] \tag{5.6}$$

By solving Eq. (5.6) for [ELP], we get

$$[ELP] = \alpha_0[ELS] + \alpha_1[E][LP] \tag{5.7}$$

where,

$$\alpha_0 = k_{13}/(k_{14} + k_{15}) \quad \text{and} \quad \alpha_1 = k_{16}/(k_{14} + k_{15}) \tag{5.8}$$

After plugging [ELP] into Eq. (5.5) and solving it for [ELS] gives

$$[ELS] = \alpha_4[LS][EE] + \alpha_5[E][LP] \tag{5.9}$$

Here α_4 and α_5 are defined in terms of individual rate constants as,

$$\alpha_4 = \frac{k_{11}(k_{14} + k_{15})}{k_{12}(k_{14} + k_{15}) + k_{13}k_{15}}$$
$$\alpha_5 = \frac{k_{14}k_{16}}{k_{12}(k_{14} + k_{15}) + k_{13}k_{15}} \tag{5.10}$$

From Eq. (5.4), [EDP] becomes

$$[EDP] = \alpha_2[EDS] + \alpha_3[E][DP] \tag{5.11}$$

$$\alpha_2 = k_7/(k_8 + k_9) \quad \text{and} \quad \alpha_3 = k_{10}/(k_8 + k_9) \tag{5.12}$$

After substituting $[EDP]$ into Eq. (5.3) and solving it [EDS], we obtain

$$[EDS] = \alpha_6[DS][EE] + \alpha_7[E][DP] \tag{5.13}$$

$$\alpha_6 = \frac{k_5(k_8 + k_9)}{k_6(k_8 + k_9) + k_7k_9} \quad \text{and} \quad \alpha_7 = \frac{k_8k_{10}}{k_6(k_8 + k_9) + k_7k_9} \tag{5.14}$$

Solution of Eq. (5.1) for [EC] is

$$[EC] = \alpha_8[E] + \alpha_9[EE] \tag{5.15}$$

$$\alpha_8 = \frac{k_{1C}}{k_2 + k_3} \quad \text{and} \quad \alpha_9 = \frac{k_{4P}}{k_2 + k_3}, k_{1C} = k_1[C] \quad \text{and} \quad k_{4P} = k_4[P] \tag{5.16}$$

By solving Eq. (5.2) for [EE] after substituting [EC] in Eq. (5.15), [EDS] in Eq. (5.13) and [ELS] in Eq. (5.9), we get

$$[EE] = \rho[E] \tag{5.17}$$

Here ρ is a function of the substrate and the product concentrations, which has the following form

$$\rho := \rho\left([DP], [LP], [DS], [LS]\right) = \frac{\Theta_1 + \Theta_2[DP] + \Theta_3[LP]}{\Theta_4 + \Theta_5[DS] + \Theta_6[LS]} \tag{5.18}$$

Here, Θ_i's $(i = 1 \ldots 6)$ are defined as

$$\begin{aligned}
\Theta_1 &= k_3\alpha_8 = \frac{k_3 k_{1C}}{k_{2P} + k_3} \\
\Theta_2 &= k_6\alpha_7 = \frac{k_6 k_8 k_{10}}{k_6(k_8 + k_9) + k_7 k_9} \\
\Theta_3 &= k_{12}\alpha_5 = \frac{k_{12} k_{14} k_{16}}{k_{12}(k_{14} + k_{15}) + k_{13} k_{15}} \\
\Theta_4 &= k_4 - k_3\alpha_9 = \frac{k_2 k_{4P}}{k_2 + k_3} \\
\Theta_5 &= k_5 - k_6\alpha_6 = \frac{k_5 k_7 k_9}{k_6(k_8 + k_9) + k_7 k_9} \\
\Theta_6 &= k_{11} - k_{12}\alpha_4 = \frac{k_{11} k_{13} k_{15}}{k_{12}(k_{14} + k_{15}) + k_{13} k_{15}}
\end{aligned} \tag{5.19}$$

After replacing $[EE]$ in Eq. (5.15) by Eqs. (5.17), (5.15) becomes

$$[EC] = (\alpha_8 + \alpha_9\rho)\,[E] \tag{5.20}$$

Substituting $[EE]$ in Eq. (5.17) into Eq. (5.13) gives

$$[EDS] = (\alpha_6[DS]\rho + \alpha_7[DP])\,[E] \tag{5.21}$$

By substituting $[EE]$ in Eq. (5.17) into Eq. (5.9), we obtain

$$[ELS] = (\alpha_4[LS]\rho + \alpha_5[LP])\,[E] \tag{5.22}$$

By plugging [ELS] in Eq. (5.22) into [ELP] in Eq. (5.7), we get

$$[ELP] = (\alpha_0\alpha_4[LS]\rho + (\alpha_0\alpha_5 + \alpha_1)\,[LP])\,[E] \tag{5.23}$$

When we replace [EE] in Eq. (5.13) by [EE] given by Eq. (5.15), we obtain

$$[EDS] = (\alpha_6[DS]\rho + \alpha_7[DP])\,[E] \tag{5.24}$$

Then substituting [EDS] in Eq. (5.24) into Eq. (5.11) gives us

$$[EDP] = (\alpha_2\alpha_6[DS]\rho + (\alpha_2\alpha_7 + \alpha_3)\,[DP])\,[E] \tag{5.25}$$

Plugging [ELP], [EDP], [ELS], [EDS], [EE] and [EC] into the conservation equation for the enzyme given by Eq. (2.1), and then solving it for [E] we get

$$[E] = \frac{[E]_0\,(\Theta_4+\Theta_5[DS]+\Theta_6[LS])}{(\Omega_1+\Omega_2[LP]+\Omega_3[DP])\,(\Theta_4+\Theta_5[DS]+\Theta_6[LS])+(\Omega_4+\Omega_5[DS]+\Omega_6[LS])\,(\Theta_1+\Theta_2[DP]+\Theta_3[LP])} \tag{5.26}$$

where,

$$\begin{aligned}
\Omega_1 &= 1+\alpha_9 > 0\\
\Omega_2 &= \alpha_1 + (1+\alpha_0)\alpha_5 > 0\\
\Omega_3 &= \alpha_3 + (1+\alpha_2)\alpha_7 > 0\\
\Omega_4 &= 1+\alpha_8 > 0\\
\Omega_5 &= \alpha_6\,(1+\alpha_2) > 0\\
\Omega_6 &= \alpha_4(1+\alpha_0) > 0
\end{aligned} \tag{5.27}$$

From Eqs. (5.25), (5.26) and (2.10), rate of change for [DP] becomes

$$\frac{d[DP]}{dt} = \frac{V_{\max 1}[DS]\,(\Theta_1+\Theta_2[DP]+\Theta_3[LP]) - V_{\max 2}[DP]\,(\Theta_4+\Theta_5[DS]+\Theta_6[LS])}{(\Omega_1+\Omega_2[LP]+\Omega_3[DP])\,(\Theta_4+\Theta_5[DS]+\Theta_6[LS])+(\Omega_4[DS]+\Omega_5+\Omega_6[LS])\,(\Theta_1+\Theta_2[DP]+\Theta_3[LP])} \tag{5.28}$$

Here $V_{\max 1}$ and $V_{\max 2}$ are defined in terms of rate constants as

$$\begin{aligned}
V_{\max 1} &= k_9\alpha_2\alpha_6[E]_0 = \frac{k_5k_7k_9}{k_6(k_8+k_9)+k_7k_9}[E]_0 > 0\\
V_{\max 2} &= (k_{10} - k_9\,(\alpha_2\alpha_7+\alpha_3))\,[E]_0 = \frac{k_6k_8k_{10}[E]_0}{k_6(k_8+k_9)+k_7k_9} > 0
\end{aligned} \tag{5.29}$$

Finally, form Eqs. (5.23), (5.26) and (2.11), rate of change for [LP] becomes

$$\frac{d[LP]}{dt} = \frac{V_{\max 3}[LS]\,(\Theta_1+\Theta_2[DP]+\Theta_3[LP]) - V_{\max 4}[LP]\,(\Theta_4+\Theta_5[DS]+\Theta_6[LS])}{(\Omega_1+\Omega_2[LP]+\Omega_3[DP])\,(\Theta_4+\Theta_5[DS]+\Theta_6[LS])+(\Omega_4[DS]+\Omega_5+\Omega_6[LS])\,(\Theta_1+\Theta_2[DP]+\Theta_3[LP])} \tag{5.30}$$

Here $V_{\max 3}$ and $V_{\max 4}$ are given by

$$V_{\max 3} = k_{15}\alpha_0\alpha_4[E]_0 = \frac{k_{11}k_{13}k_{15}}{k_{12}(k_{14}+k_{15})+k_{13}k_{15}}[E]_0 > 0$$
$$V_{\max 4} = (k_{16} - k_{15}\,(\alpha_0\alpha_5+\alpha_1))\,[E]_0 = \frac{k_{12}k_{14}k_{16}[E]_0}{k_{12}(k_{14}+k_{15})+k_{13}k_{15}} > 0 \tag{5.31}$$

References

1. L. Keszthelyi, Q. Rev. Biophys. **28**(4), 473 (1995)
2. D. Fitz, H. Reiner, K. Plankensteiner, B.M. Rode, Curr. Chem. Biol. **1**, 41 (2007)
3. P.G.H. Sandars, Int. J. Astrobiol. **4**(1), 49 (2005)
4. C.H. Senanayake, D. Krishnamurthy, I. Gallou, in *Handbook of Chiral Chemicals*, ed. by D. Ager (CRC Press, Boca Raton, FL, 2005)
5. B.S. Sekhon, Int. J. PharmTech. Res. **2**(2), 1584 (2010)
6. W.J.M. Hegeman, R.W.P.M. Laane, Rev. Environ. Contam. Toxicol. **73**, 85 (2002)
7. K.M.J. Brands, A.J. Davies, Chem. Rev. **106**, 2711 (2006)
8. J. Bojarski, H.Y. Aboul-Enein, A. Ghanem, Curr. Anal. Chem. **1**, 59 (2005)
9. K. Soai, T. Kawasaki, Top. Curr. Chem. **284**, 1 (2008)
10. K. Soai, T. Kawasaki, Chem. Today **27**(6), 3 (2009)
11. N.J. Turner, Curr. Opin. Chem. Biol. **8**, 114 (2004)
12. J.A. Arcos, C.G. Hill Jr, C. Otero, Biotechnol. Bioeng. **73**(2), 104 (2001)
13. W.R. Berendsen, G. Gendrot, S. Resnick, M. Reuss, J. Biotechnol. **121**, 213 (2006)
14. J. Xiong, J. Wu, G. Xu, L. Yang, Chem. Eng. J. **138**(1–3), 258 (2008)
15. G.D. Yadav, I.V. Borkar, Ind. Eng. Chem. Res. **47**(10), 3358 (2008)
16. I.H. Segel, *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems* (Wiley, New York, 1975)
17. X. Wang, W. Li, V. Kumar, J. Cell. Plast. **45**, 353 (2009)
18. K.B. Taylor, *Enzyme Kinetics and Mechanisms* (Kluwer, Dordrecht, 2002), pp. 2–3
19. W.R. Berendsen, A. Lapin, M. Reuss, Chem. Eng. Sci. **62**, 2375 (2007)
20. N. Yildirim, N. Ankaralioglu, D. Yildirim, F. Akcay, Appl. Math. Comput. **137**(1), 67 (2003)
21. N. Yildirim, J. Math. Chem. **32**(3), 271 (2002)
22. A. Aydemir, *Modeling of Enzyme Catalyzed Racemic Reactions and Modification of Enantioselectivity*, PhD. Thesis (Institut für Technische Chemie, Gottfried Wilhelm Leibniz Universität, 2010)
23. C.S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Sih, J. Am. Chem. Soc. **104**, 1294 (1982)
24. C.S. Chen, S.H. Wu, G. Girdaukas, C.J. Sih, J. Am. Chem. Soc. **109**(9), 2812 (1987)
25. C.S. Chen, C.J. Sih, Angew. Chemie Int. Ed. Eng. **28**(6), 695 (1989)
26. W.R. Berendsen, G. Gendrot, A. Freund, Biotechnol. Bioeng. **95**(5), 883 (2006)
27. H. Hirata, G. Kondo, K. Kawauchi, Y.G. Chen, K. Sakaki, H. Yanagishta, J. Oleo. Sci. **55**(5), 239 (2006)
28. S. Sahin, J. Warnå, P. Maki-Arvela, T. Salmi, D.Y. Murzin, J. Chem. Technol. Biotechnol. **85**, 192 (2009)