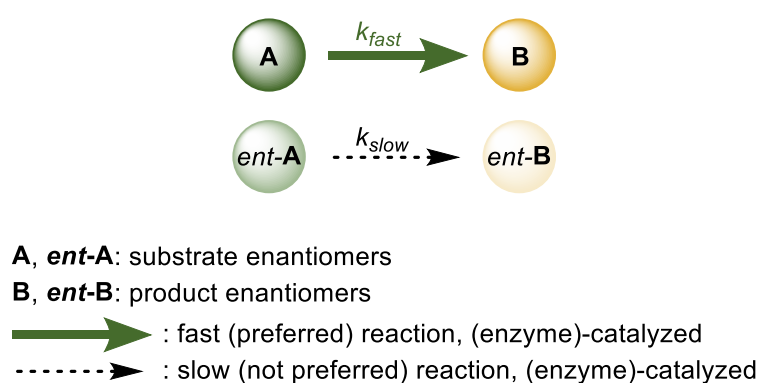# ENANTIO — SELECTIVITY

a Computer Program for the Determination of the Enantioselectivity (*E*-Value) in the Kinetic Resolution of Enantiomers

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A, ***ent*-A**: substrate enantiomers
B, ***ent*-B**: product enantiomers
⟶ : fast (preferred) reaction, (enzyme)-catalyzed
------▶ : slow (not preferred) reaction, (enzyme)-catalyzed

Scheme 1: Kinetic resolution and the required input parameters.

When a racemic substrate (**A** and *ent*-**A**) is subjected to an enzymatic reaction (e.g. hydrolysis), chiral discrimination of the enantiomers occurs [1,2]. The same applies to any asymmetric catalytic reaction.

Due to the chiral environment of the active site of the enzyme, one enantiomer fits better into the active site than its counterpart and is therefore converted at a higher rate, a process termed kinetic resolution. To ensure a high enantio-selectivity, the difference in the reaction rates of the individual enantiomers (k_{fast}, k_{slow}) should be as large as possible. In an ideal case it is so extreme that the 'good' enantiomer is transformed quickly while the other remains untouched. Then the enzymatic reaction will cease at 50% conversion, when all of the more reactive enantiomer (**A**) is selectively converted to **B**. Hence, after separation, each of the enantiomers (**B** and remaining *ent*-**A**) can be obtained in 50% yield.

In practice, however, the difference in - or better the ratio of - the reaction rates of enantiomers is not infinite, but measurable. What one observes in this case, is not a complete standstill of the reaction at 50% conversion but a marked decrease in reaction rate at around this point and one encounters some crucial dependencies:

— Since the substrate enantiomers (**A**, *ent*-**A**) are converted at different rates, their ratio does not remain constant during the reaction, but varies with the degree of conversion.

— As a consequence, the optical purity of both substrate (e.e.$_S$) and product (e.e.$_P$) becomes a *function of the conversion.*

A very useful treatment of the kinetics of enzymatic resolution, describing the dependency of the conversion (c) and the enantiomeric excess of substrate (e.e.$_S$) and product (e.e.$_P$), was developed by C. J. Sih in 1982 [3] on a theoretical basis laid by K. B. Sharpless [4] and K. Fajans [5]. The parameter describing the enantio-selectivity of a kinetic resolution was introduced as the dimensionless 'Enantiomeric Ratio' (*E*) [3, 6], i. e. the ratio of the relative (second order) rate constants of enantiomers ($E = k_{fast}/k_{slow}$), which remains constant throughout the reaction and is determined by the environment of the system, i.e. the substrate, enzyme, solvent, temperature, pH, etc. The *E*-value is a synonym for the so-called 'selectivity factor' (*s*) [7]. Whereas *E* is used more often in biocatalysis, the *s*-factor is more common in chemo-catalysis. In mathematical terms, however, both are identical.

For irreversible reactions, such as an ester hydrolysis in an aqueous medium or acyl transfer reactions (using enol esters or acid anhydrides as acyl donors), the enantio-selectivity of the reaction - expressed as the Enantiomeric Ratio (*E*) - can be mathematically linked to the conversion (c) of the reaction, and the optical purities of substrate (e.e.$_S$) and product (e.e.$_P$) [8].

The dependence of the enantioselectivity and the conversion of the reaction is

for the product: for the substrate:

$$E = \frac{\ln[1-c(1+e.e._P)]}{\ln[1-c(1-e.e._P)]} \qquad E = \frac{\ln[(1-c)(1-e.e._S)]}{\ln[(1-c)(1+e.e._S)]}$$

c = conversion, e.e.$_S$, e.e.$_P$ = enantiomeric excess of substrate (S, i.e. **A** + *ent*-**A**) and product (P, i.e. **B** + *ent*-**B**); E = Enantiomeric Ratio [8].

The above mentioned equations give reliable results except for very low and very high extents of conversion, where accurate measurement is restricted by errors derived from sample manipulation. In such cases, the following equation is recommended instead, because only values for the optical purities of substrate and product need be measured. The latter are *relative* quantities in contrast to the conversion, which is an *absolute* quantity [8].

$$E = \frac{\ln \dfrac{[e.e._P(1-e.e._S)]}{(e.e._P + e.e._S)}}{\ln \dfrac{[e.e._P(1+e.e._S)]}{(e.e._P + e.e._S)}}$$

Based on the equations above, the program ENANTIO can be used for the calculation of the enantioselectivity (E-value) of kinetic resolutions taking two values out of three, i.e. enantiomeric excess of substrate (e.e.$_S$), product (e.e.$_P$) and/or conversion (c). The E-Value serves as (conversion-independent) constant for the determination of the enantio-selectivity of a kinetic resolution. As a rule of thumb, Enantiomeric Ratios below 15 are unacceptable for practical purposes. They can be regarded as moderate to good from 15-30, and above this value they are excellent. It must be emphasized that values of E >200 cannot be accurately determined due to inaccuracies emerging from the determination of the enantiomeric excess (e.g. by HPLC, GC or NMR), because in this range even a very small variation of e.e.$_S$ or e.e.$_P$ causes a significant change in the numerical value of E.

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