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Supporting Information

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Rapid determination of both the activity and enantioselectivity of ketoreductases

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General Experimental:

Commercial grade reagents and solvents were purchased from Sigma-Aldrich and used without further purification. The ketoreductase (KRED) enzymes and NADPH were purchased from Biocatalytics Inc. (Pasadena, CA). All enzymes were purchased in lyophilized form. The (*R)*-alcohol oxidase was developed in-house and is internally designated oxidase M_{3-5}.

Reaction conversion for the conventional screening was monitored using reverse phase high performance liquid chromatography (HPLC) at 210 nm using an Agilent 1100 series HPLC and a Zorbax Eclipse XDB-C18 (50 x 4.6 mm) column with a flow rate of 1 mL/min (60% acetonitrile / 40% water) for 10 minutes. Enantiomeric excess for the conventional screening was determined by normal phase high performance liquid chromatography (HPLC) at 210 nm using an Agilent 1100 series HPLC and a Chiralpak OD-H (250 x 4.6 mm) column with a flow rate of 1 mL/min (95% hexanes / 5% 2-propanol) for 20 minutes. Specific rotation of the phenylethanol product was established by comparison to literature.[1]

Conventional Screening Conditions:

Conventional screening reactions were run at 1 mL scale in 100 mM potassium phosphate buffer using the following conditions and concentrations: 30 ºC, pH 7.0, 2 g/L ketoreductase (KRED) enzyme, 2 g/L glucose dehydrogenase (GDH), 20 g/L glucose, 1 g/L NADP cofactor, 10 g/L acetopohenone. 2 mg of ketoreductase enzyme, 2 mg of glucose dehydrogenase enzyme, 20 mg of glucose and 1 mg of NADP cofactor were added to 1 mL of 100 mM potassium phosphate buffer (pH 7.0). 10 mg of acetophenone was then added to the reaction. The reactions were run in 2 mL Eppendorf tubes and placed in a shaking, temperature controlled incubator (Thermomixer) at 30 ºC. 100 uL samples were taken every 4 hours. Samples for reverse phase HPLC were diluted 1:10 with acetonitrile, filtered and run using the method described above. Samples for normal phase HPLC were extracted with methyl tertbutyl ether (MTBE), dried down, re-suspended in the mobile phase (95% hexanes / 5% 2-propanol), and run according to the method described above.

Dual Wavelength Screening Conditions:

Dual wavelength ATRUee screening reactions were run at 0.1 mL scale in 100 mM potassium phosphate buffer using the following conditions and concentrations: 30 ºC, pH 7.0, 1 g/L ketoreductase (KRED) enzyme, 4 g/L alcohol oxidase, 2 U/uL horse radish peroxidase, 0.5 mM ABTS, 0.15 mM NADPH cofactor, 2 mM acetopohenone. To a 100 mM potassium phosphate buffer solution containing the ABTS dye, NADPH cofactor and acetophenone substrate was added the ketoreductase enzyme. Absorbance was monitored at 340 nm using a 96 well plate spectrophotometer. The ketoreductase activity was determined based on the initial rate of absorbance change at 340 nm. Upon complete consumption of the NADPH, the horse radish peroxidase and alcohol oxidase were added

to the reaction. ABTS radical cation formation as a result of hydrogen peroxide formation was tracked at 400nm. The enantioselectivity of the ketoreductase was determined using equations 1-4. The total amount of conversion to *R+S* enatiomers of alcohol product was calculated based on the amount of NADPH consumed (eq 1). The amount of *R*-alcohol was determined based on the amount of ABTS cation formed (eq 2). The amount of *S*-alcohol is calculated as the difference of eq 1 and eq 2 (eq 3). Finally, the ee is calculated (eq 4).

$[(R)\text{-}2]+[(S)\text{-}2] = [\text{NADPH}]_o\text{-}[\text{NADPH}]_f \rightarrow$ at 340 nm (eq 1)

$[(R)\text{-}2] = [\text{ABTS cation}]/2 \rightarrow$ at 400 nm (eq 2)

$[(S)\text{-}2] = [\text{NADPH}]_o\text{-}[\text{NADPH}]_f - [\text{ABTS cation}]/2$ (eq 3)

$$ee = \frac{[(R)-2]-[(S)-2]}{[(R)-2]+[(S)-2]} \qquad \text{(eq 4)}$$

Activity Trajectory for the Rapid Understanding of ee (ATRUee) Screening Conditions:

A kinetic model was created to predict the reaction progress of enzymes with a wide range of enantioselectivities. The model was created in Excel using an iterative calculation process, whereby the (*R*)-alcohol product of the ketone reduction was rapidly converted back to ketone substrate by the alcohol oxidase. The ketone was then continually reduced by the ketoreductase, consuming the NADPH cofactor, until the reaction proceeded to completion. Starting concentrations, rate constants and enzyme selectivities were input into the kinetic model, and the model was used to calculate the new concentrations at a small increment forward in time. These new concentrations were then fed back into the model to predict the next set of concentrations for the next increment of time. In this way, a kinetic model of the reaction progress for enzymes of varying selectivities was generated. The equations used to model the assay reaction system along with an example of the iterative Excel spreadsheet for an enzyme with a selectivity value E = 4 is shown below, followed by the predicted reaction profiles for a range of selectivities.

sec	mM	g/L	mM	mM	mM	g/L	mM	g/L	AU	mM
time	**[S]**	**[E KRED]**	**[P1] S**	**[P2] R**	**NADPH**	**[E Rao]**	**[H2O2]**	**[E hrp]**	**abs 340nm**	**NADPH**
0.00	0.15	1.00	0.00	0.00	1.50	5.00	0.00	5.00	2.73	0.00
1.00	0.15	1.00	0.00	0.00	1.50	5.00	0.00	5.00	2.72	0.00
2.00	0.15	1.00	0.00	0.00	1.49	5.00	0.01	5.00	2.72	0.01
3.00	0.14	1.00	0.00	0.01	1.49	5.00	0.01	5.00	2.71	0.01
4.00	0.14	1.00	0.00	0.01	1.49	5.00	0.01	5.00	2.71	0.01
5.00	0.14	1.00	0.00	0.01	1.49	5.00	0.01	5.00	2.71	0.01
6.00	0.14	1.00	0.00	0.01	1.48	5.00	0.02	5.00	2.70	0.02
7.00	0.14	1.00	0.00	0.01	1.48	5.00	0.02	5.00	2.70	0.02
8.00	0.13	1.00	0.00	0.02	1.48	5.00	0.02	5.00	2.69	0.02
9.00	0.13	1.00	0.00	0.02	1.48	5.00	0.02	5.00	2.69	0.02
10.00	0.13	1.00	0.01	0.02	1.47	5.00	0.03	5.00	2.68	0.03
11.00	0.13	1.00	0.01	0.02	1.47	5.00	0.03	5.00	2.68	0.03
12.00	0.13	1.00	0.01	0.02	1.47	5.00	0.03	5.00	2.67	0.03
13.00	0.12	1.00	0.01	0.03	1.47	5.00	0.03	5.00	2.67	0.03
14.00	0.12	1.00	0.01	0.03	1.46	5.00	0.04	5.00	2.67	0.04
15.00	0.12	1.00	0.01	0.03	1.46	5.00	0.04	5.00	2.66	0.04
16.00	0.12	1.00	0.01	0.03	1.46	5.00	0.04	5.00	2.66	0.04
17.00	0.12	1.00	0.01	0.03	1.46	5.00	0.04	5.00	2.65	0.04
18.00	0.11	1.00	0.01	0.04	1.46	5.00	0.04	5.00	2.65	0.04
19.00	0.11	1.00	0.01	0.04	1.45	5.00	0.05	5.00	2.65	0.05
20.00	0.11	1.00	0.01	0.04	1.45	5.00	0.05	5.00	2.64	0.05
21.00	0.11	1.00	0.01	0.04	1.45	5.00	0.05	5.00	2.64	0.05
22.00	0.11	1.00	0.01	0.04	1.45	5.00	0.05	5.00	2.63	0.05
23.00	0.11	1.00	0.01	0.04	1.45	5.00	0.05	5.00	2.63	0.05
24.00	0.11	1.00	0.01	0.04	1.44	5.00	0.06	5.00	2.63	0.06
25.00	0.10	1.00	0.01	0.05	1.44	5.00	0.06	5.00	2.62	0.06
26.00	0.10	1.00	0.01	0.05	1.44	5.00	0.06	5.00	2.62	0.06
27.00	0.10	1.00	0.01	0.05	1.44	5.00	0.06	5.00	2.62	0.06
28.00	0.10	1.00	0.01	0.05	1.44	5.00	0.06	5.00	2.61	0.06
29.00	0.10	1.00	0.01	0.05	1.43	5.00	0.07	5.00	2.61	0.07
30.00	0.10	1.00	0.01	0.05	1.43	5.00	0.07	5.00	2.61	0.07
31.00	0.09	1.00	0.01	0.06	1.43	5.00	0.07	5.00	2.60	0.07
32.00	0.09	1.00	0.01	0.06	1.43	5.00	0.07	5.00	2.60	0.07
33.00	0.09	1.00	0.01	0.06	1.43	5.00	0.07	5.00	2.60	0.07
34.00	0.09	1.00	0.01	0.06	1.43	5.00	0.07	5.00	2.60	0.07
35.00	0.09	1.00	0.02	0.06	1.42	5.00	0.08	5.00	2.59	0.08
36.00	0.09	1.00	0.02	0.06	1.42	5.00	0.08	5.00	2.59	0.08
37.00	0.09	1.00	0.02	0.06	1.42	5.00	0.08	5.00	2.59	0.08
38.00	0.09	1.00	0.02	0.06	1.42	5.00	0.08	5.00	2.58	0.08

K KRED	1.3E-02
K KRED1	2.5E-03
K KRED2	1.0E-02
K R ao	1.0E-01
K HRP	1.0E-01
	per h
Kd KRED	0
Kd R ao	0
Kd HRP	0
ABS	1
E	4
ee alcohol	60.0

General equation of enzyme activity over time assuming first order irreversible deactivation:

$$[E](t) = [E]_0 e^{-k_d (t - t_0)} \quad \textbf{(1)}$$

General rate constant equation:

$$k(T) = Ae^{\left(\frac{-E_a}{RT}\right)} \quad \textbf{(2)}$$

General rate equation:

$$r = k(T)[f(C_1, C_2, C_3 ...)] \quad \textbf{(3)}$$

Equations for KRED and AO enzyme activity over time:

$$[E_{KRED}](t) = [E_{KRED}]_0 e^{-k_{d\,KRED} (t - t_0)} \quad \textbf{(4)}$$

$$[E_{AO}](t) = [E_{AO}]_0 e^{-k_{d\,AO} (t - t_0)} \quad \textbf{(5)}$$

Rate of *S*-product formation:

$$r_{P1} = k_{KRED1}[S][NADPH][E_{KRED}] \quad \textbf{(6)}$$

Rate of *R*-product formation:

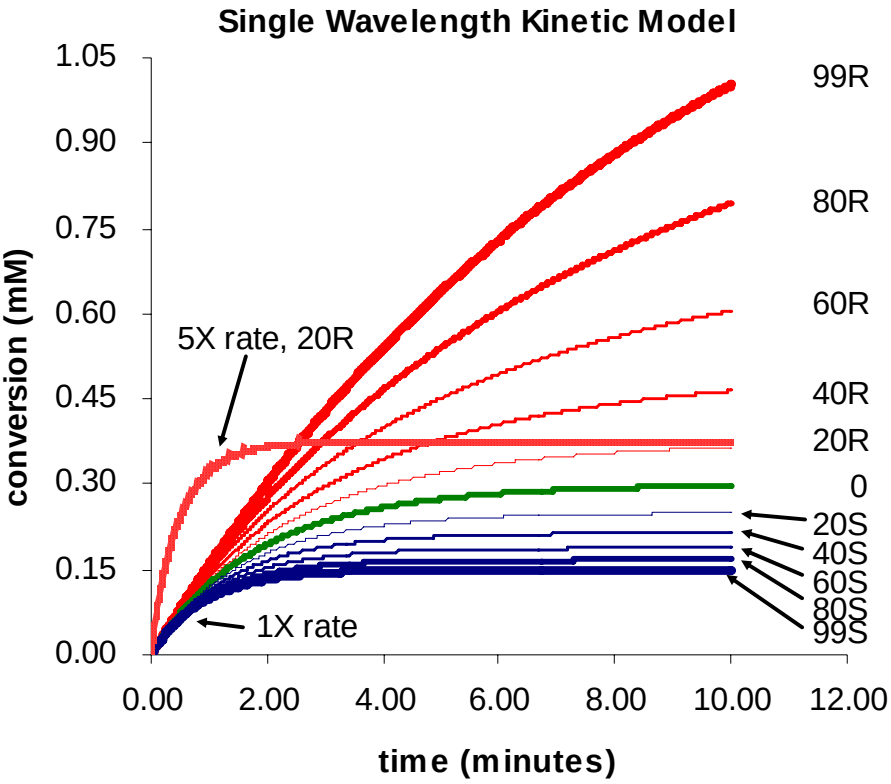
$$r_{P2} = k_{KRED2}[S][NADPH][E_{KRED}] - k_{AO}[P2][O_2][E_{AO}] \quad \textbf{(7)}$$

Rate of NADPH consumption:

$$-r_{NADPH} = k_{KRED1}[S][NADPH][E_{KRED}] + k_{KRED2}[S][NADPH][E_{KRED}] \quad \textbf{(8)}$$

Rate of substrate consumption:

$$-r_S = r_{P1} + r_{P2} = k_{KRED1}[S][NADPH][E_{KRED}] + k_{KRED2}[S][NADPH][E_{KRED}] - k_{AO}[P2][O_2][E_{AO}] \quad \textbf{(9)}$$



Single wavelength ATRUee screening reactions were run at 0.1 mL scale in 100 mM potassium phosphate buffer using the following conditions and concentrations: 30 ºC, pH 7.0, 1 g/L ketoreductase (KRED) enzyme, 4 g/L alcohol oxidase, 1.5 mM NADPH cofactor, 0.15 mM acetophenone. To a 100 mM potassium phosphate buffer solution (pH 7.0) containing the alcohol oxidase, NADPH cofactor and acetophenone substrate was added the ketoreductase. The reactions were run in a 96 well plate and monitored using a well plate spectrophotometer. NADPH consumption was tracked at 340 nm until the reaction reached completion. The ketoreductase activity was determined based on the initial rate of absorbance change at 340 nm. The endpoint of the reaction was compared to the kinetic model to determine the e.e.

Phenylethanol Standard Normal Phase Chiral Assay Chromatogram: