**Supplementary information**

# Kinetic model of asymmetric dehydration of aldoxime catalyzed by immobilized OxdPsp in organic solvent

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## 1 Eperimental information

2-phenylpropionaldehyde, hydroxylamine hydrochloride, sodium carbonate, cyclohexane, ethyl acetate mercaptoethanol, dipotassium hydrogen phosphate and n-heptane were purchased from Shanghai Macklin Biochemical Technology Co., Ltd.

GF254 plates (Qingdao Ocean Chemical Co., Ltd, China) were employed in thin-layer chromatography (TLC) with cyclohexane/ethyl acetate. 2-Phenylpropanenitrile was stained with phosphomolybdic acid for visualizing.

**HPLC Analysis**

(*R*)-2-Phenylpropanenitrile ((*R*)-**2**), (*S*)-**2**, (*Z/S*)-2-Phenylpropanal oxime ((*Z/S*)-**1**), (*E/S*)-**1**, (*Z/R*)-**1** and (*E/R*)-**1** were determined with HPLC (SHIMADZULC-16A) assembled a chiral column (CHIRALPAK IB-N3 column, 245×4.6mm, 3μm) and a diode array detector（SPD-M20）operated at 210 nm. The mobile phase was the mixture of 97 hexane, 3 isopropanol and 0.1 trifluoroacetic acid (v/v) at 1.0 ml $min^{-1}$at 20 °C. According to the order of retention time, from first to last, the chromatographic peaks for the above substrates and products are as follows: (*R*)-**2**, (*S*)-**2**, (*Z/S*)-**1**, (*E/S*)-**1**, (*Z/R*)-**1** and (*E/R*)-**1**.

**NMR spectra**

The $^{1}H$ and $^{13}C$ NMR spectra of 2-Phenylpropanal oxime and 2-Phenylpropanenitrile were measured in $CDCl_3$ by a Bruker AV-500 instrument at 500 MHz for $^{1}H$ and 125 MHz for $^{13}C$, respectively. The unit of δ and coupling constants are in ppm and Hz, respectively.

## 2 Synthesis of *E/Z*-2-Phenylpropionaldehyde oxime *via* condensation of 2-phenylpropionaldehyde with hydroxylamine hydrochloride.

*E/Z*-2-Phenylpropionaldehyde oxime (**1**) was prepared as described in literature[1]. *ac*-2-phenylpropionaldehyde (16.1 g, 120 mmol) was added into the suspension of hydroxylamine hydrochloride (12.5 g, 180 mmol, 1.5 eq.) and sodium carbonate (19.08g, 180 mmol, 1.5 eq.) suspended in deionized water and the mixture was vigorously stirred at room temperature more than 2 hours until conversion was completed, monitoring according to TLC detection. After three extractions of the reaction solution with ethyl acetate (equal volume ratio), the organic phases combined were dried over $MgSO_4$ and filtered. The solvent filtered was evaporated out under reduced pressure at 10℃ to suppress isomerization and the resulting crude product was purified *via* silica column chromatography, yielding the colorless oily product with a ratio of 9:1 of *E* to *Z*). E/Z-isomer of (*E/Z*)-2-phenylpropanal oxime was separated *via* the P230PII flash chromatography system (Elite Analytical Instruments Co., Ltd, Dalian, China) with cyclohexane/ethyl acetate as mobile phase, producing (*E/Z*)-2-Phenylpropanal oxime with 97/3 of *E/Z*. The conformation and *E/Z* of the product were assayed by $^{1}H$-NMR and the enantiomeric excess (ee) was assayed by HPLC assembled a chiral column.

*E-rac*-2- Phenylpropanal oxime:

**$^{1}H$ NMR**: δ 7.51 (d, *J* = 6.0 Hz, 1H, *H*C=NOH), 7.39 – 7.20 (m, 5H, Ar-*H*), 3.68 (m, *J* = 6.8 Hz, 1H, C*H*-CH=NOH), 1.47 (d, *J* = 7.0 Hz, 3H, C$H_3$).

**$^{13}$C NMR**: δ 155.10, 142.12, 128.73, 127.38, 126.96, 40.37, 18.76

Both the $^{1}$H and $^{13}$C NMR data recorded for (R)-**3** were consistent with the literature values[2].

### 3 Culture of cells harboring OxdPsp

The gene of OxdPsp bound to pRSFDuet-1 was synthesized by Beijing Haichuang Keye Biotechnology Co., Ltd and the constructed expression plasmid pRSF-OxdPsp is capable to directly used for transforming.

The vector pRSF-OxdPsp was transformed into *E. coli* BL21 (DE3) cells and the screened cells harboring the OxdPsp were inoculated in 150 mL Terrific Broth (TB) medium including 50 μg/mL kanamycin in a 1000 mL Erlenmeyer flask in a constant temperature shaker with 200 rpm at 37℃. When OD600 of the cultivated broth was up to 0.6-0.8, IPTG was added to a concentration of 0.5 mM for inducing the expressing the recombinant OxdPsp at 18 ℃ for approx. 20 hours. The cells bearing the OxdPsp were harvested using a high speed frozen centrifuge GL-21M by Xiangyi Centrifuge Instrument Co., Ltd (8000 rpm, 10 min, 4 °C) and washed twice with 50 mM phosphate buffer solution with pH 7.0 carrying phenylmethylsulfonyl fluoride (0.1 mM)，NaCl (300 mM), glycerol (5%, v/v) and mercaptoethanol (8 mM) and centrifuged to yield cell pellets. The washed cell pellets were resuspended in the phosphate buffer solution and disrupted in an ice bath using a sonicator operating for 10 seconds, pausing for 15 seconds, and lasting for 30 cycles (Ultrasonic cell breaker JY92-II, Ningbo Life Technology Co., Ltd, China). The resulting in fragmented cell broth was storage at 4℃ for subsequent use.

### 4 Activity assay for the immobilized aldoxime dehydratase

The activity assay for the immobilized aldoxime dehydratase (Oxd) was carried out in a approx. 20 ml glass vial (inner diameter 22 mm, height 57 mm) with cap, the vial was placed horizontally on a constant temperature shaker, which oscillates back and forth instead of rotating. The reaction mixture 6 ml (0.05M in solvent n-heptane) was given the vial under argon atmosphere at 150 rpm at 30 °C for 15min for preheating, and a certain amount of the immobilized Oxd was added into the vial and the reaction mixture was incubated at 150 rpm at 30 °C for 10 min. The immobilized Oxd was immediately removed by simple filtration from the mixture to quench the reaction and a aliquot was taken out for HPLC assay. One unit (U) of the immobilized Oxd is defined as the amount of the required enzyme to catalyze substrate aldoxime **1** dehydrating to form 1 μmol of the corresponding nitrile, 2-phenylpropanenitrile (**2),** per min at 30 °C and the calculation formula for the activity of the immobilized Oxd is as follows,

$$\text{Specific activity} = C_n/C_e t \quad (\text{U/mg})$$

Where $C_n$, $C_e$, and t were the concentration of **2** (μM/ml), the concentration of the immobilized Oxd (mg/ml), and incubation time (10 min), respectively.

## 5 HPLC spectra

==== Shimadzu LabSolutions Analysis Report ====

Sample Name : SP/purified240103
Injection Volume : 20 uL
HPLC :SHIMADZU LC-16
Column name :CHIRALPAK IB N-3
Mobile phase :n-Hexane / Isopropyl alcohol/ trifluoroacetic acid=93 / 7 / 0.14
Wave length :UV 209nm

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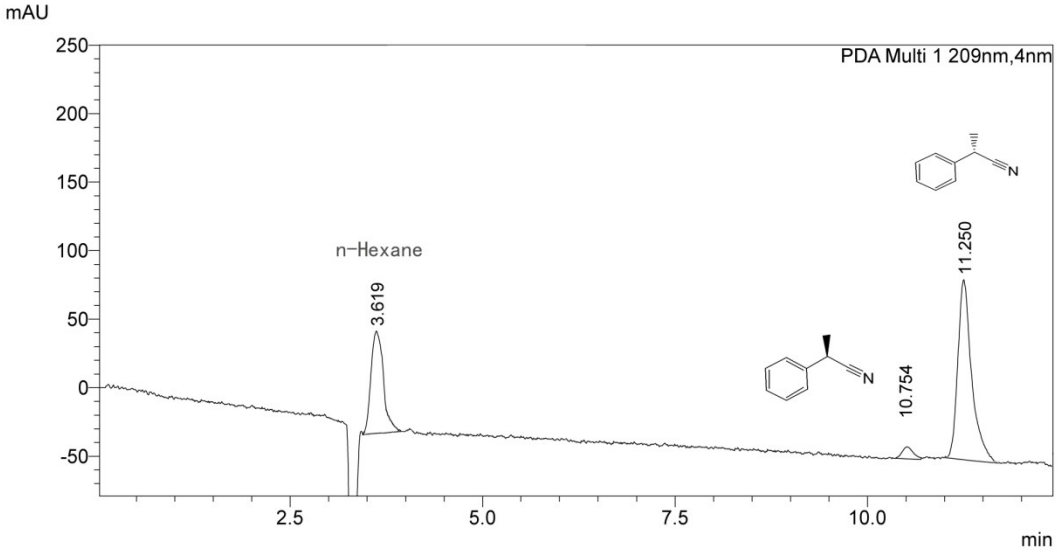


<Peak Table>

PDA Ch1 209nm

| Peak# | Ret. Time | Area | Height | area% | Height% |
|---|---|---|---|---|---|
| 1 | 3.619 | 666583 | 67991 | 28.447 | 31.448 |
| 2 | 10.754 | 48378 | 17641 | 2.065 | 8.159 |
| 3 | 11.250 | 1628269 | 130572 | 69.488 | 60.393 |

Fig. S1 HPLC spectrum for (*S*)-2-Phenylpropanenitrile ((*S*)-**2**) and (*R*)-2-Phenylpropanenitrile ((*R*)-**2**).

==== Shimadzu LabSolutions Analysis Report ====

Sample Name : SP/purified240101
Injection Volume : 20 uL
HPLC :SHIMADZU LC-16
Column name :CHIRALPAK IB N-3
Mobile phase :n-Hexane / Isopropyl alcohol/ trifluoroacetic acid=97 / 3 / 0.06
Wave length :UV 209nm

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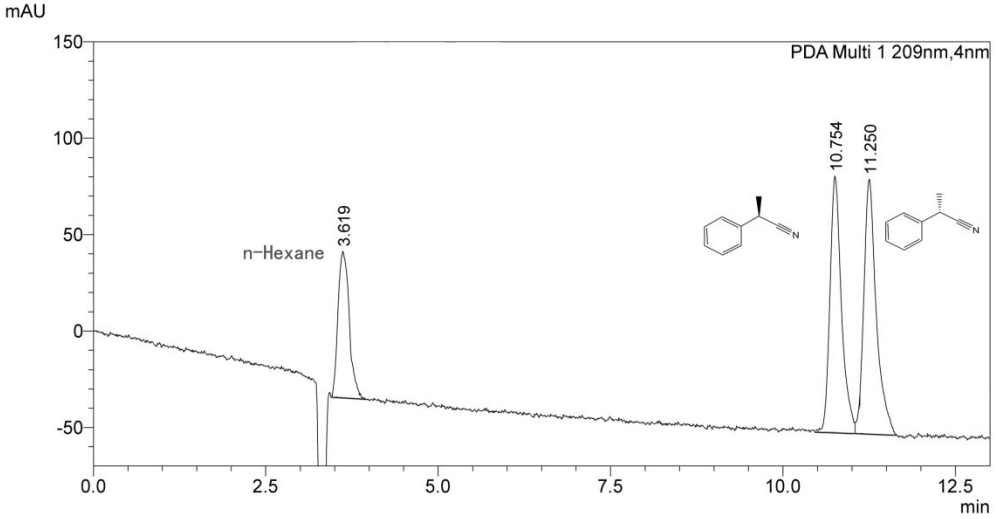


<Peak Table>

PDA Ch1 209nm

| Peak# | Ret. Time | Area | Height | area% | Height% |
|---|---|---|---|---|---|
| 1 | 3.619 | 618839 | 65092 | 16.454 | 19.792 |
| 2 | 10.754 | 1569287 | 132755 | 41.725 | 40.366 |
| 3 | 11.250 | 1572908 | 131034 | 41.821 | 39.842 |

Fig. S2 HPLC spectrum for racemic 2-Phenylpropanenitrile((*S*) -**2** and (*R*)-**2**).

## 5 References


[1] R. Metzner, S. Okazaki, Y. Asano, H. Gröger, Cyanide-free enantioselective synthesis of nitriles: Synthetic proof of a biocatalytic concept and mechanistic insights, ChemCatChem. 6 (2014) 3105–3109. https://doi.org/10.1002/cctc.201402612.

[2] H. Gröger, Y. Asano, Cyanide-Free Enantioselective Catalytic Strategies for the Synthesis of Chiral Nitriles, J. Org. Chem. 85 (2020) 6243–6251. https://doi.org/10.1021/acs.joc.9b02773.