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Supporting Information

Bio-electrocatalytic Alkene Reduction Using Ene-Reductases with Methyl Viologen as Electron Mediator

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Supplementary Information

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Zheng Wei,[a] Tanja Knaus,[a] Matteo Damian,[a] Yuxin Liu,[a,b] Cássia S. Santana,[a] Ning Yan,[c] Gadi Rothenberg*[a] and Francesco G. Mutti*[a]

[a] Van 't Hoff Institute for Molecular Sciences, HIMS-Biocat, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, The Netherlands

[b] Department of Biomolecular Systems, Max Planck Institute for Colloids and Interfaces, 14476 Potsdam, Germany

[c] Key Laboratory of Artificial Micro- and Nano-Structures of Ministry of Education, School of Physics and Technology, Wuhan University, Wuhan 430072, China

* Corresponding authors

E-mail: F.Mutti@uva.nl; G.Rothenberg@uva.nl;

Table of Contents

1. Experimental section

1.1. Materials and equipment

Methyl viologen dichloride hydrate ($MVCl_2$), KCl and KH_2PO_4 were purchased from Merck (Darmstadt, Germany). K_2HPO_4 and NaCl were bought from Roth. 2-Cyclohexen-1-one, Cyclohexanone, 2,6,6-trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone), *trans*-2-methyl-2-pentenal, 2-methylpentanal, 2-methyl-2-cyclohexen-1-one and 2-methylcyclohexanone were purchased form Sigma. All solutions were prepared using deionized water. The phosphate buffer solution (PBS) contains 0.1 M KCl and 10 mM phosphate buffer (pH = 7.4).

Cyclic voltammetry (CV), and chronoamperometry were obtained by the Reference 600 electrochemical station (Gamry Instruments Inc, USA). A three-electrode system was made up of a carbon paper electrode (CT Carbon Cloth with a Microporous Layer) as the working electrode, an Ag/AgCl electrode (saturated KCl) as the reference electrode, and a Pt wire as the counter electrode. The h-type bottle was designed and made by the Technology Centre at the University of Amsterdam. The Gas chromatography flame ionization detector (GC-FID) analysis was done by 7890B GC system (Agilent Technologies, USA). The anaerobic conditions were obtained by operating the experiment in the glovebox (Belle Technology, UK Ltd).

1.2. Expression and purification of the enzyme

The ene reductases, PETNR (*Enterobacter cloacae*) and TOYE (*Thermoanaerobacter pseudethanolicus*), have been expressed and purified as described in our previous publication[1]. All proteins were stored as concentrated stocks in KPi buffer (50 mM, pH 7.0).

1.3. Preparation of the electrolyte solution

All the electrolyte solutions were prepared in pH 7.4 KPi, 100 mM buffer supplemented with 0.1 M KCl. Then, all solutions were purged with N_2 for at least 2 h. The concentration of MV^{2+} for bio-electrocatalytic reduction was 1 mM in pH 7.4 KPi, 100 mM buffer supplemented with 0.1 M KCl. Then, all the electrolyte solutions were stored in the glove-box one day before use.

1.4. Pretreatment of the electrodes and the proton exchange membrane

The proton exchange membrane (Nafion® 117) was pretreated by heating it in Milli-Q water at 80 °C for 1 hour, followed by immersion in 3% H_2O_2 at 80 °C for another hour. Subsequently, the membrane was heated again in Milli-Q water at 80 °C for 3 hours, followed by 1 hour of heating in 1 M H_2SO_4 at 80 °C. Finally, the membrane underwent a 3-hour heating in Milli-Q water at 80 °C. The pretreated exchange membranes were stored in Milli-Q water before use.

The carbon paper electrodes were pretreated by scanning through 10 cycles from -1 V to 1 V vs Ag/AgCl in 0.1 M HCl solution by using the CV technique. Ag/AgCl and Pt wire were used as the reference and the counter electrodes, respectively. The CVs were recorded at a scan rate of 50 mV/s.

The proton exchange membrane (Nafion® 117) was pretreated by heating it in Milli-Q water (>18.2 MΩcm, TOC<5 ppb) at 80 °C for 1 hour, followed by immersion in 3% H2O2 at 80 °C for another hour. Subsequently, the membrane was heated again in Milli-Q water at 80 °C for 3 hours, followed by 1 hour of heating in 1 M H2SO4 at 80 °C. Finally, the membrane underwent a 3-hour heating in Milli-Q water at 80 °C. The pretreated exchange membranes were stored in Milli-Q water before use.

1.5. CV of MV^{2+} solution with enzyme and substrate

The reaction system was assembled and did the CV tests in the glove-box . In the anodic part, the solution was pH 7.4, 100 mM KPi buffer with 0.1 M KCl. In the cathodic part, the solution was 1 mM MV^{2+} in pH 7.4, 100 mM KPi buffer with 0.1 M KCl. The working electrode employed in this study was a carbon paper electrode with a geometric surface area of 0.5 cm^2. The CVs

were recorded in the potential range between -0.475 V and -0.9 V *vs* Ag/AgCl, based on the reference articles.[2] The scan rate was 10 mV/s. After finished the blank MV^{2+} solution test, 100 µL of a 1 mM stock solution of PTENR was added into the cathodic chamber. Then, 100 µL of a 1 M stock solution of cyclohex-2-en-1-one was added into the cathodic chamber. The scan rate, potential range, and the cycle numbers were always the same in those three experiments.

1.6. CA of MV^{2+} solution with enzyme and substrate

In this experiment, the geometric surface area of carbon paper electrode was 1.5 cm^2 (1 cm × 1.5 cm). The i-t tests were conducted under a constant potential of -0.9 V *vs* Ag/AgCl. 100 µL of a stock solution of 1 mM PETNR was added at the beginning of the reaction, as shown in the curve labeled 'with PETNR.' After 7200 s into the reaction, 100 µL of a stock solution of 1 M cyclohex-2-en-1-one was added in the cathodic chamber. The entire reaction lasted for 14400 s (4 h). The conditions for both curves were identical, differing only in the presence or absence of enzymes.

1.7. Calculation of faradaic efficiency

The faradaic efficiency was calculated by following the equation:

$$\text{faradaic efficiency (FE)} = \frac{n \times c \times V \times F}{I \times t} \times 100\%$$

n is the number of electrons transferred in our reaction system; c is the concentration of the product; V is the volume of the reaction solution; F is the Faraday constant (96485 C mol^{-1}); I is the average current during the reaction; t is the reaction time.

1.8. Conversion of the substrate with and without PETNR

The pretreated exchange membranes and electrodes were assembled into the h-type bottle system within the glove-box. A solution containing 100 µL of a stock solution of 1 mM PETNR and 100 µL of a stock solution of 1 M cyclohex-2-en-1-one was added into 10 mL of 1 mM MV^{2+}, dissolved in 100 mM pH 7.4 KPi buffer with 100 mM KCl, before initiating the electrochemical reaction. Operating under a constant -0.9 V *vs* Ag/AgCl reduction potential, the reaction was carried out for 14400 s (4 h). The conversion curves were measured by gas chromatography-flame ionization detector (GC-FID) analysis using an Agilent DB1701-30m column.

1.9. Optimization of the reaction time

The pretreated exchange membranes and electrodes were assembled into the h-type bottle system within the glove-box. A solution containing 100 µL of a stock solution of 1 mM PETNR and 100 µL of a stock solution of 1 M cyclohex-2-en-1-one was added into 10 mL of 1 mM MV^{2+}, dissolved in 100 mM pH 7.4 KPi buffer with 100 mM KCl, before initiating the electrochemical reaction. Operating under a constant -0.9 V *vs* Ag/AgCl reduction potential, the reaction was carried out for 21600 s (6 h).

Over the 6-hour reaction duration, 200 µL of the reaction solution from the cathodic chamber was extracted every hour. Following the sixth hour, a final reaction was initiated and lasted for 21600 s. Post-reaction, an additional 200 µL of the solution were collected. All these reaction solutions were prepared for subsequent GC-FID analysis, with conversions measured using the DB1701-30m column. This entire process was repeated three times for reliable results.

1.10. Bioelectrocatalytic hydrogenation of different substrates

The pretreated exchange membranes and electrodes were assembled into the h-type bottle system within the glove-box. A solution containing 100 µL of a stock solution of 1 mM PETNR and 100 µL of a stock solution of 1 M substrates was introduced into 10 mL of 1 mM MV^{2+}, dissolved in 100 mM pH 7.4 KPi buffer with 100 mM KCl, before initiating the electrochemical reaction.

Operating under a constant -0.9 V reduction potential *vs* Ag/AgCl, the reaction was carried out for 14400 s (4 h).

All those product yield results were calculated based on the conversion rate of substrate under GC-FID analysis by using the DB1701-30m column (30 m, 250 µm, 0.25 µm).

Method A: DB1701-30m-A: constant pressure 6.9 psi, split ratio 40:1, T injector 250 °C. Temperature program: T initial 60 °C, hold 6.5 min, gradient 20 °C/min up to 100 °C; hold 1 min, gradient 20 °C/min up to 280 °C; hold 1 min.

The enantiomeric excess for ketoisophorone was measured on a Chirasil Dex CB column (25 m, 0.32 mm, 0.25 µm);

Method B: Chirasil-Dex-CB: Constant Flow: 1.4 mL/min, split ratio 10:1, T injector 200 °C. Temperature program: T initial 100 °C, hold 2 min, gradient 2.5 °C/min up to 120 °C; hold 10 min, gradient 5 °C/min up to 180 °C; hold 2 min[3].

The enantiomeric excess for 2-methyl-2-cyclohexen-1-one was measured on a Restek Rt-ßDEXsa column (30m, 0.25 mm, 0.25 µm);

Method C: Restek Rt- ßDEXsa: Constant flow: 0.9 mL/min, split ratio 10:1, T injector 200 °C. Temperature program: T initial 80 °C, hold 10 min, gradient 4 °C/min up to 120 °C, hold 2 min, gradient 20 °C/min up to 180 °C, hold 1 min[1].

The enantiomeric excess for 2-methyl-2-pentenal was measured on a Restek Rt-ßDEXsa column (30 m, 0.25 mm, 0.25 µm); split ratio 10: 1; injector 180 °C, detector 200 °C, flow 1 mL/min; Temp. program: 80 °C hold 10 min; 4 °C/min to 120 °C hold 2 min; 20 °C/min to 180 °C hold 1 min.[4]

Method C: Restek Rt- ßDEXsa: Constant flow: 0.9 mL/min, split ra 10:1, T injector 200 °C. Temperature program: T initial 80 °C, hold 10 min, gradient 4 °C/min up to 120 °C, hold 2 min, gradient 20 °C/min up to 180 °C, hold 1 min[1]

2. Supplementary Figures

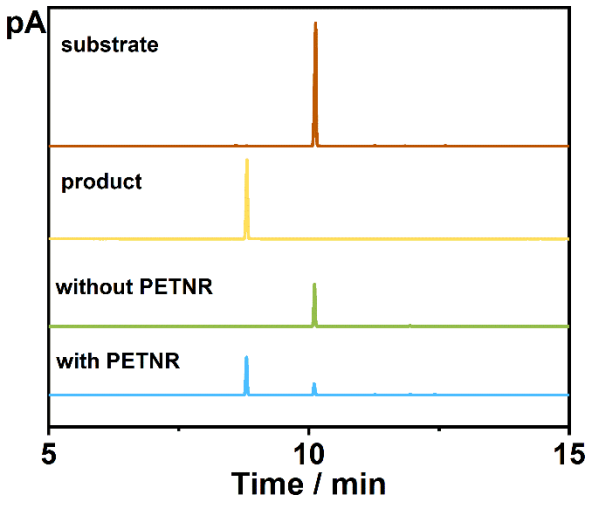

Figure S1. GC-FID chromatograms of reference substrate cyclohexanone (brown), reference product cyclohex-2-en-1-one (yellow). Bio-electrocatalytic hydrogenation without PETNR (green) and with PETNR (blue). The reaction solution contained 10 µM PETNR, 1 mM MV^{2+} in 0.1 M pH=7.4 KPi buffer supplemented with 0.1 M KCl. The concentration of cyclohex-2-en-1-one was 10 mM.

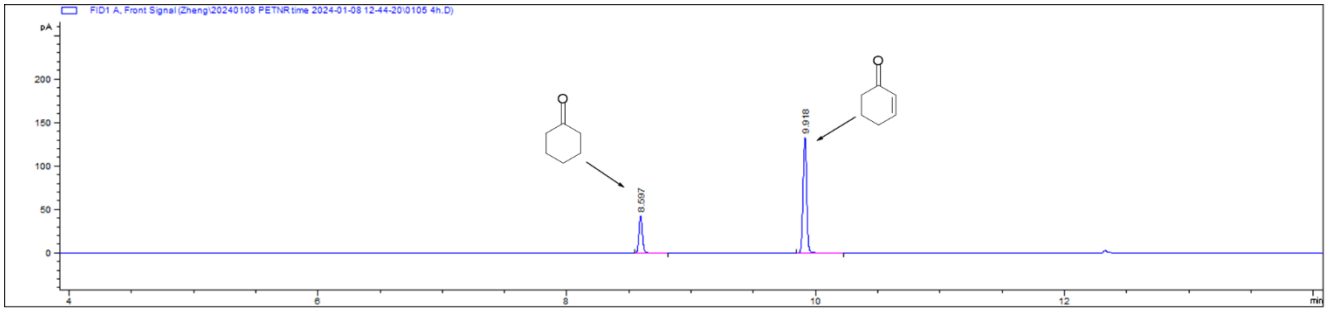

Figure S2. GC-FID chromatograms for the determination of the conversion of 2-cyclohexen-1-one catalyzed by PETNR.

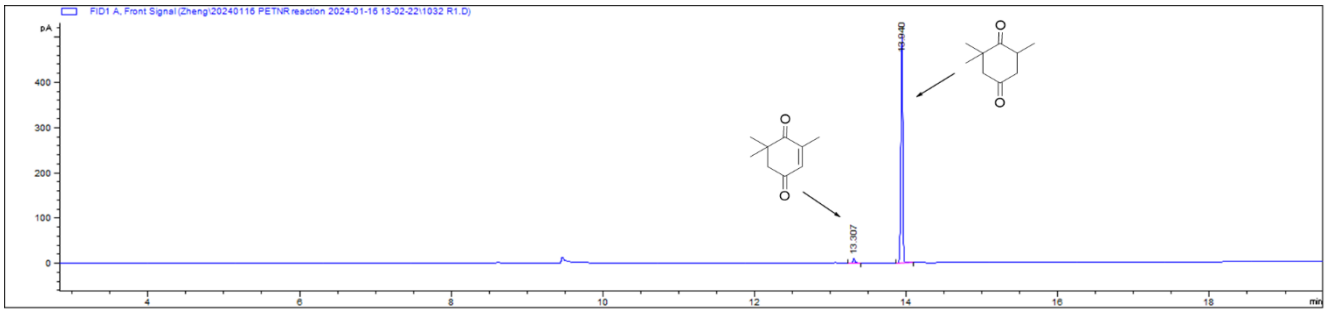

Figure S3. GC-FID chromatograms for the determination of the conversion of ketoisophorone catalyzed by PETNR.

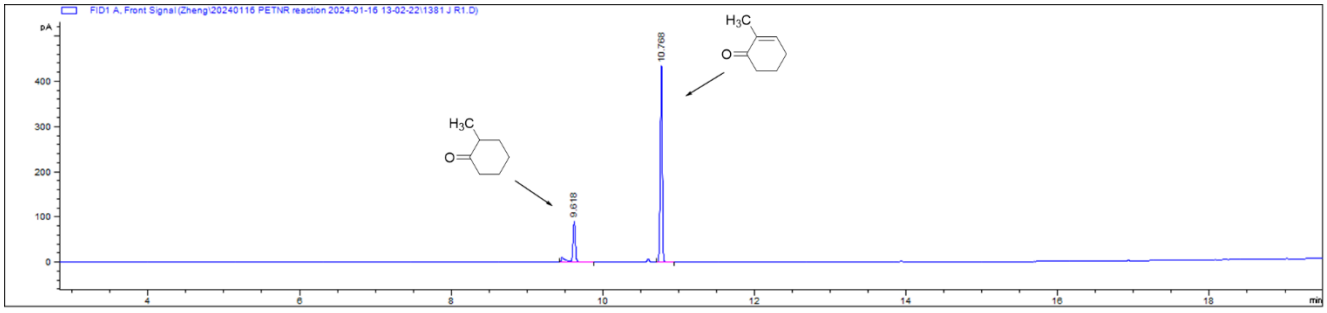

Figure S4. GC-FID chromatograms for the determination of the conversion of 2-methyl-2-cyclohexen-1-one catalyzed by PETNR.

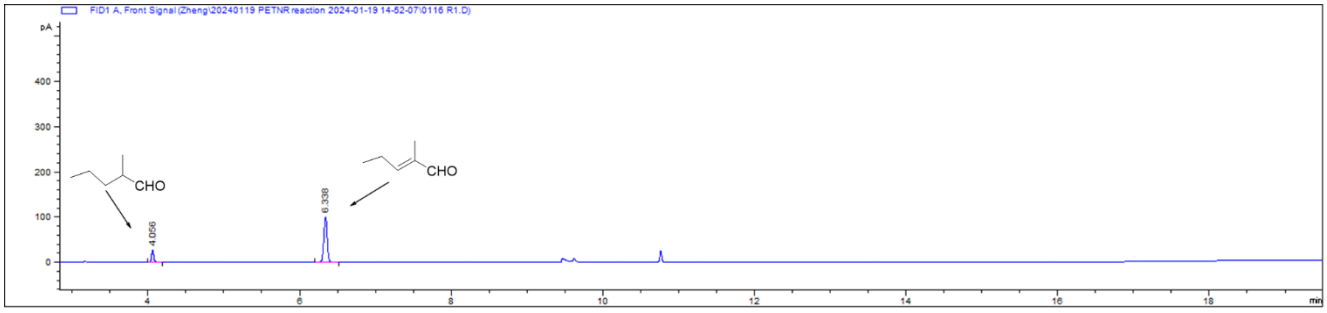

Figure S5. GC-FID chromatograms for the determination of the conversion of 2-methyl-2-pentenal catalyzed by PETNR.

Figure S6. GC-FID chromatograms for the determination of the conversion of 2-cyclohexen-1-one catalyzed by TOYE.

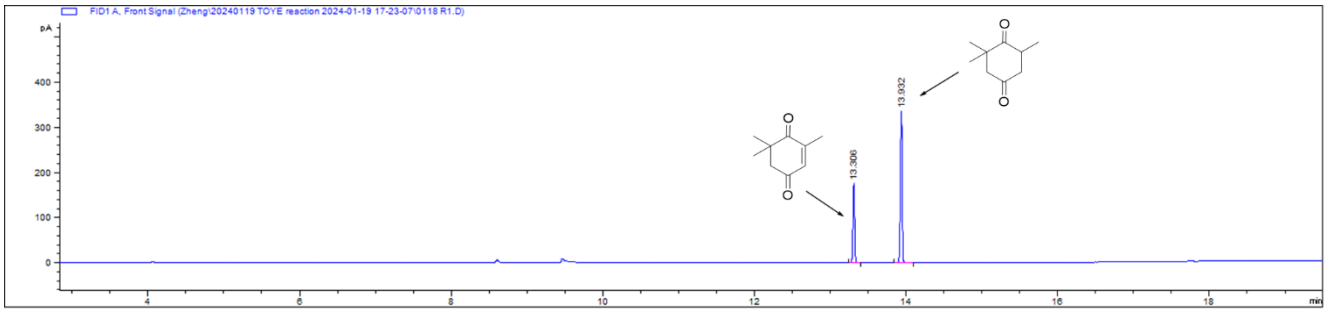

Figure S7. GC-FID chromatograms for the determination of the conversion of ketoisophorone catalyzed by TOYE.

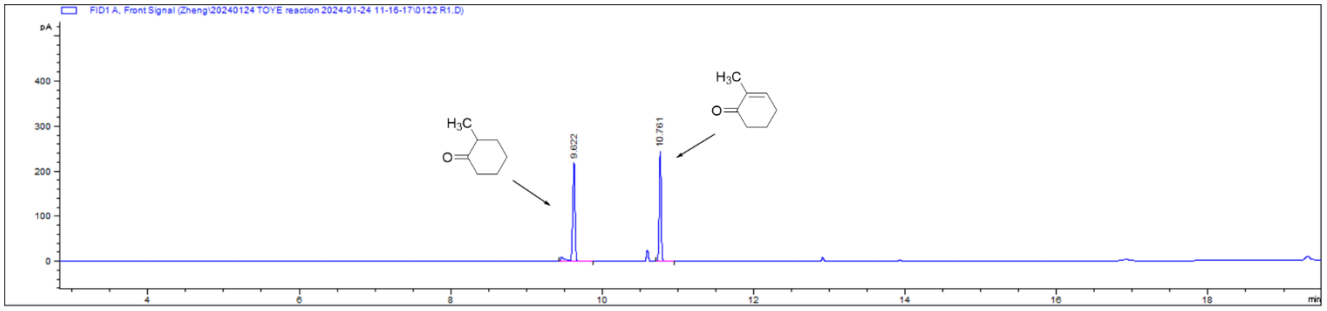

Figure S8. GC-FID chromatograms for the determination of the conversion of 2-methyl-2-cyclohexen-1-one catalyzed by TOYE.

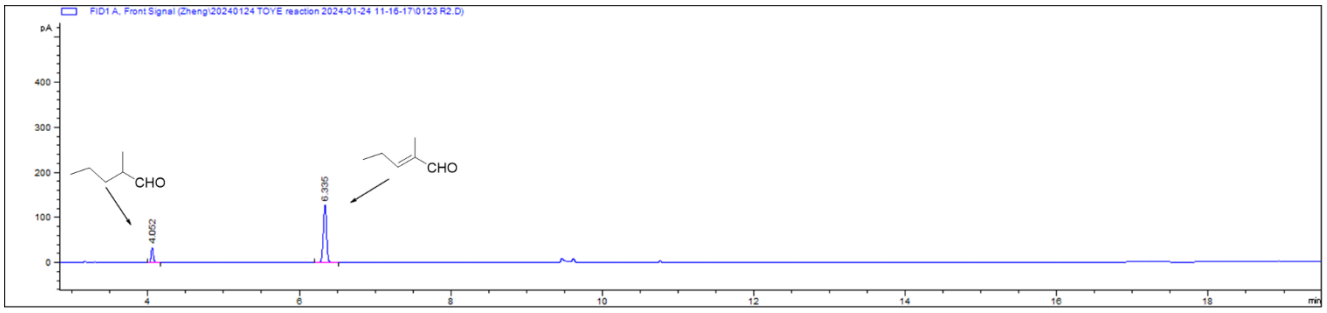

Figure S9. GC-FID chromatograms for the determination of the conversion of 2-methyl-2-pentenal catalyzed by TOYE.

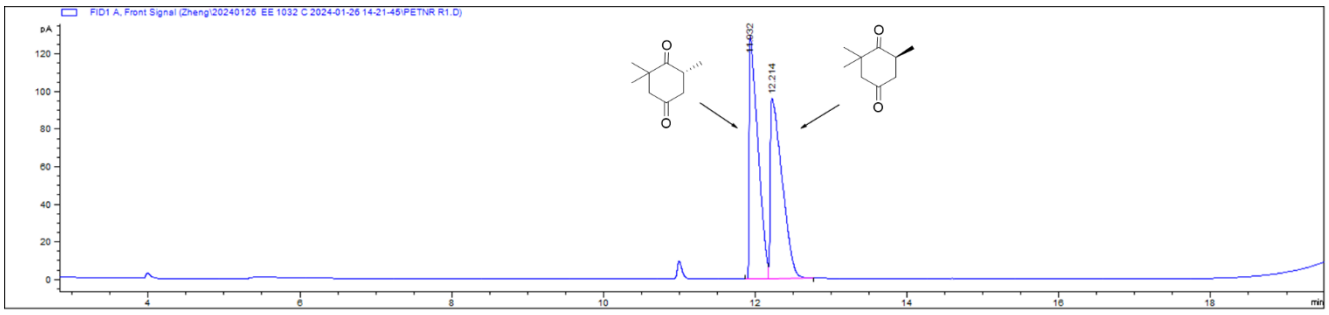

Figure S10. GC-FID chromatograms for the determination of the enantiomeric excess of ketoisophorone catalyzed by PETNR.

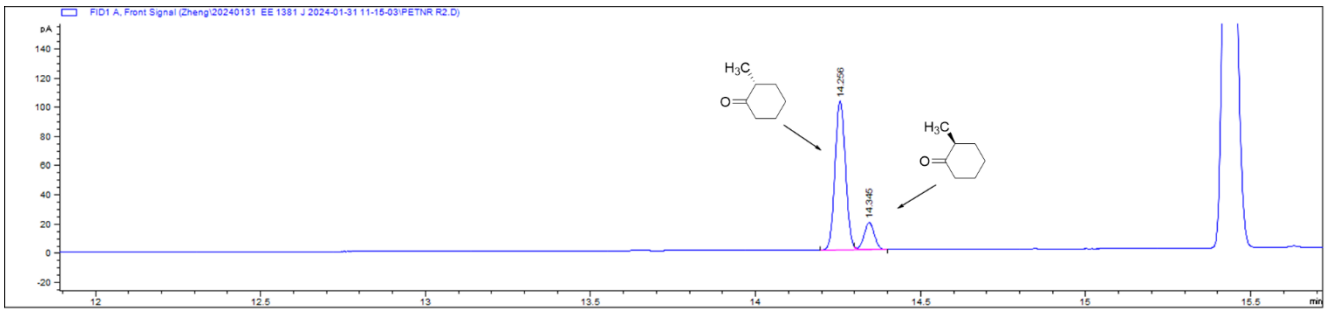

Figure S11. GC-FID chromatograms for the determination of the enantiomeric excess of 2-methyl-2-cyclohexen-1-one catalyzed by PETNR.

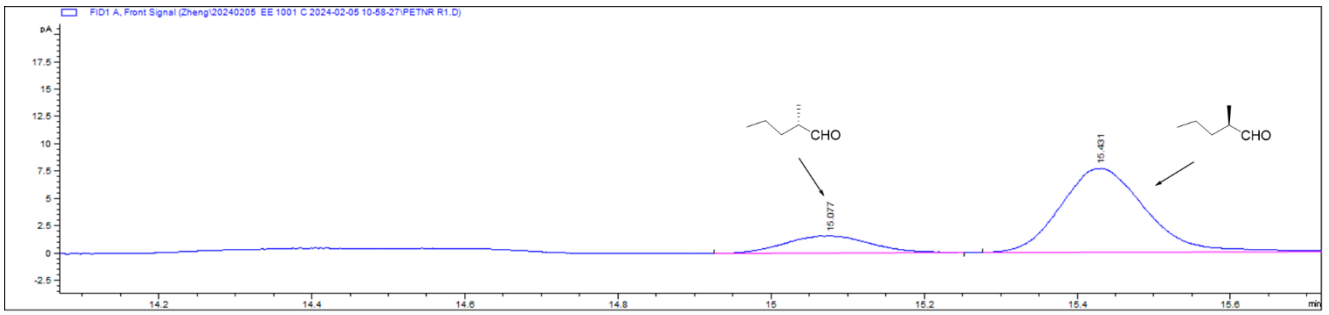

Figure S12. GC-FID chromatograms for the determination of the enantiomeric excess of 2-methyl-2-pentenal catalyzed by PETNR.

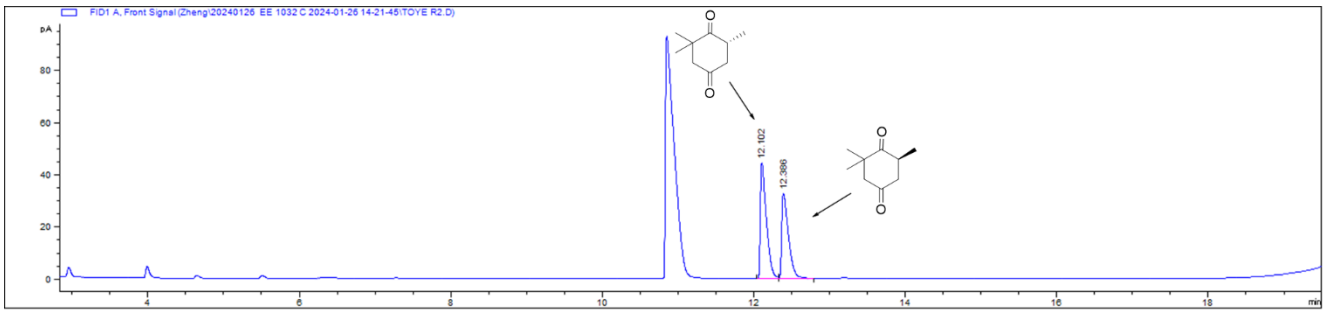

Figure S13. GC-FID chromatograms for the determination of the enantiomeric excess of ketoisophorone catalyzed by TOYE.

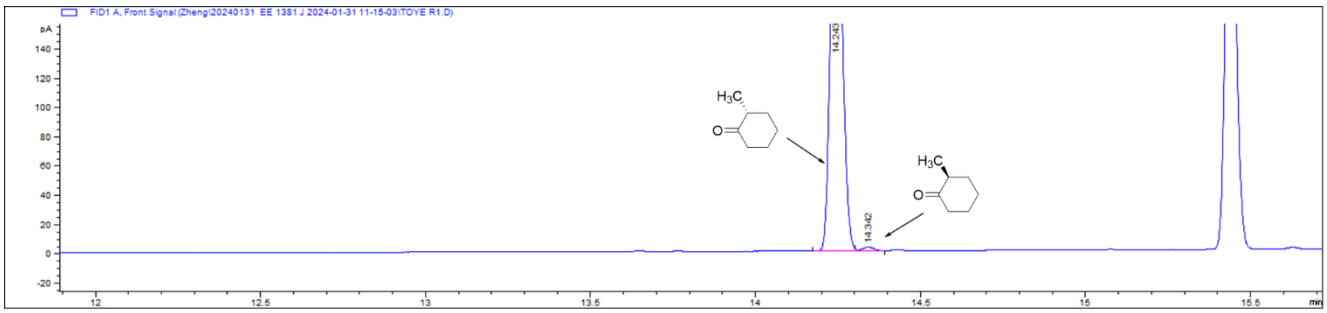

Figure S14. GC-FID chromatograms for the determination of the enantiomeric excess of 2-methyl-2-cyclohexen-1-one catalyzed by TOYE.

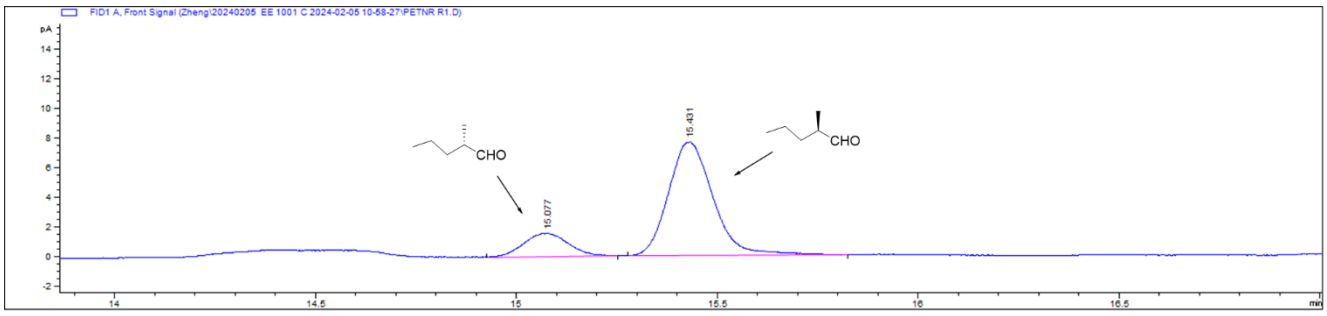

Figure S15. GC-FID chromatograms for the determination of the enantiomeric excess of 2-methyl-2-pentenal catalyzed by TOYE.

References

[1] T. Knaus, M. L. Corrado, F. G. Mutti, *ACS Catal* **2022**, *12*, 14459-14475.

[2] H. Chen, R. Cai, J. Patel, F. Dong, H. Chen, S. D. Minteer, *J. Am. Chem. Soc.* **2019**, *141*, 4963-4971.

[3] T. Knaus, C. E. Paul, C. W. Levy, S. de Vries, F. G. Mutti, F. Hollmann, N. S. Scrutton, *J. Am. Chem. Soc.* **2016**, *138*, 1033-1039.

[4] T. Knaus, F. G. Mutti, L. D. Humphreys, N. J. Turner, N. S. Scrutton, *Org. Biomol. Chem.* **2015**, *13*, 223-233.