



Article

2-Ketoglutarate-generated in vitro enzymatic biosystem facilitates Fe(II)/2-ketoglutarate-dependent dioxygenase-mediated C–H bond oxidation for (2*s*,3*r*,4*s*)-4-Hydroxyisoleucine synthesis

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1. SDS-PAGE analysis of purified enzymes

The recombinant enzymes were purified by His-Trap HP affinity chromatography using a His-Trap HP affinity column, then the high salt content of the purified fractions was removed using a disposable PD-10 desalting column.

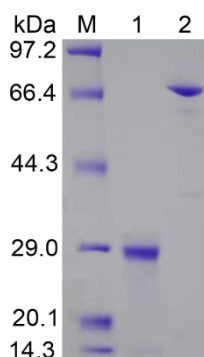


Figure S1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis of purified enzymes: M: Molecular weight standards (Bio-Rad); lane 1: Purified L-isoleucine dioxygenase (IDO); lane 2: Purified L-glutamate oxidase (LGOX).

2. Effects of pH and temperature on enzyme activity

To determine the effects of pH on enzyme activity, we separately measured the IDO and LGOX activity in the standard reaction mixture at a 4.0–9.0 pH range. The effects of temperature on IDO and LGOX activity were evaluated by incubating a standard reaction mixture for 10 min at temperatures ranging from 10–45 °C. Figure S2A shows that IDO and LGOX activity were at a maximum at 30 °C. Therefore, the enzyme cascade temperature was set to 30 °C. Figure S2B shows IDO and LGOX activity with respect to pH and buffer. IDO had maximum activity in Tris-HCl buffer (pH 7.0), whereas LGOX had 93% activity in Tris-HCl buffer (pH 8.0). IDO had long-term stability at 25–35 °C (Figure 2C) while the relative LGOX activity was >90% after 3 h incubation at 25 °C and 30 °C. However, LGOX was unstable at 35 °C (Fig. 2D). Thus, Tris-HCl buffer (pH 7.0) and 30 °C were applied in one-pot 4-HIL production.

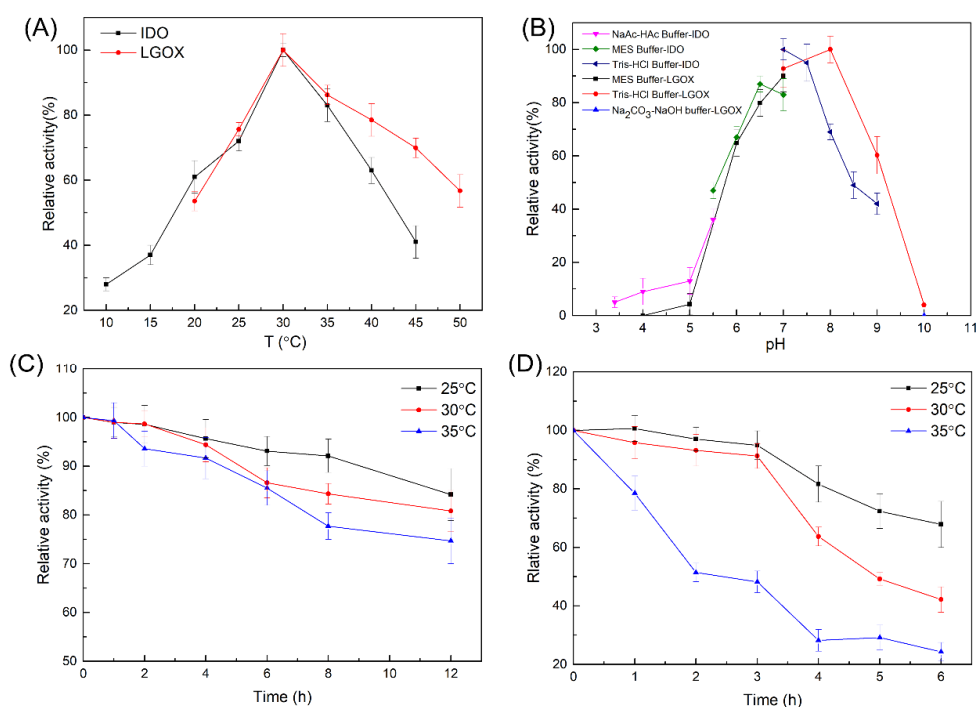
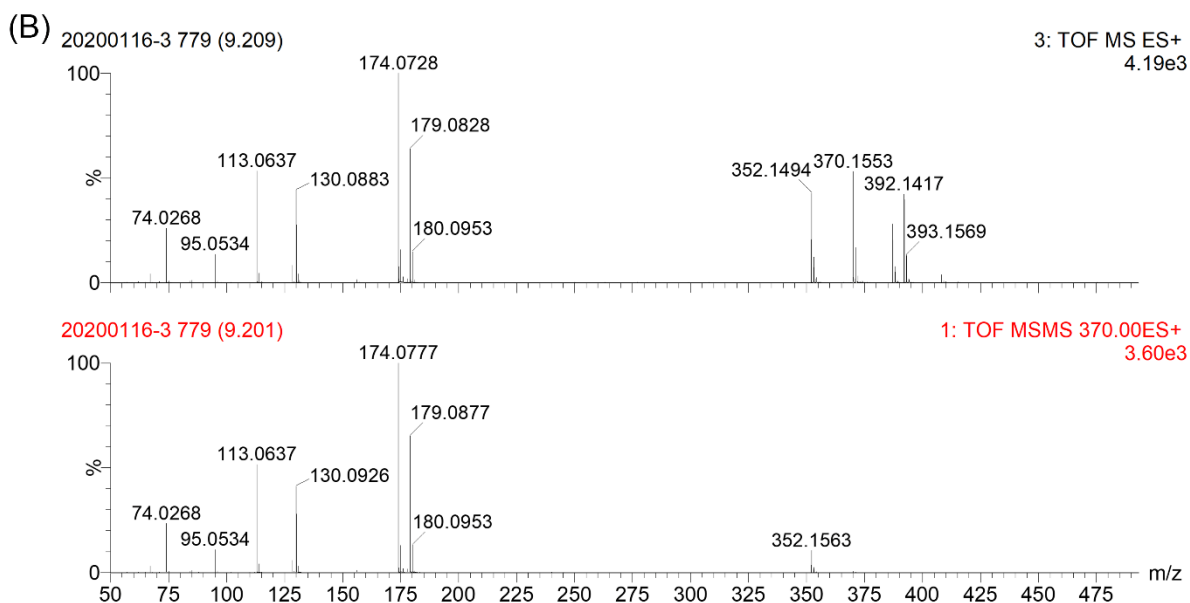
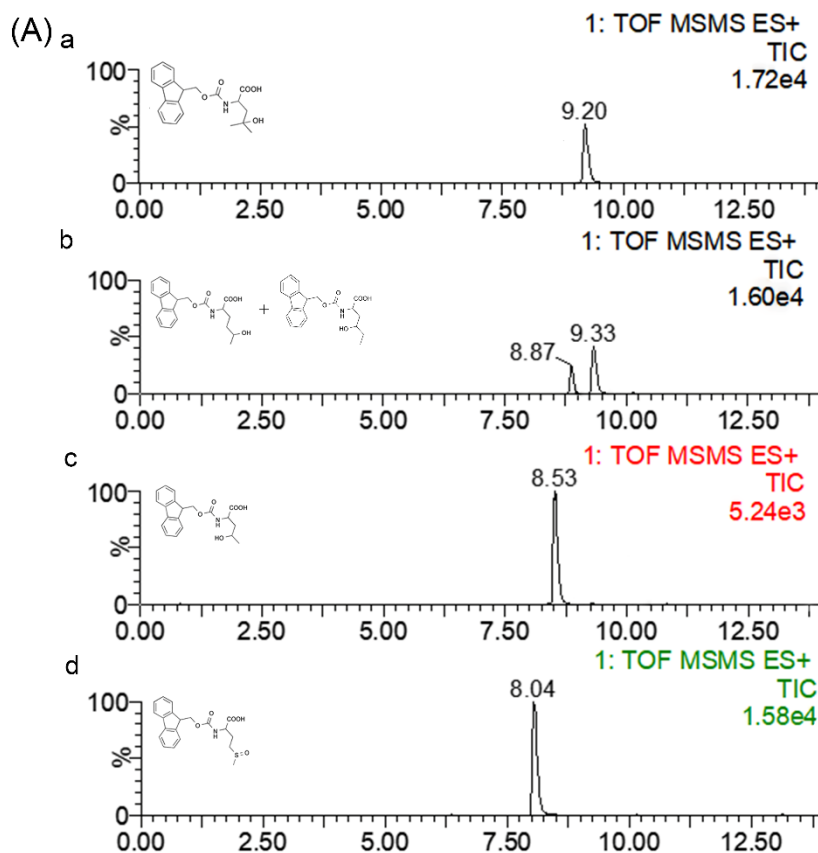
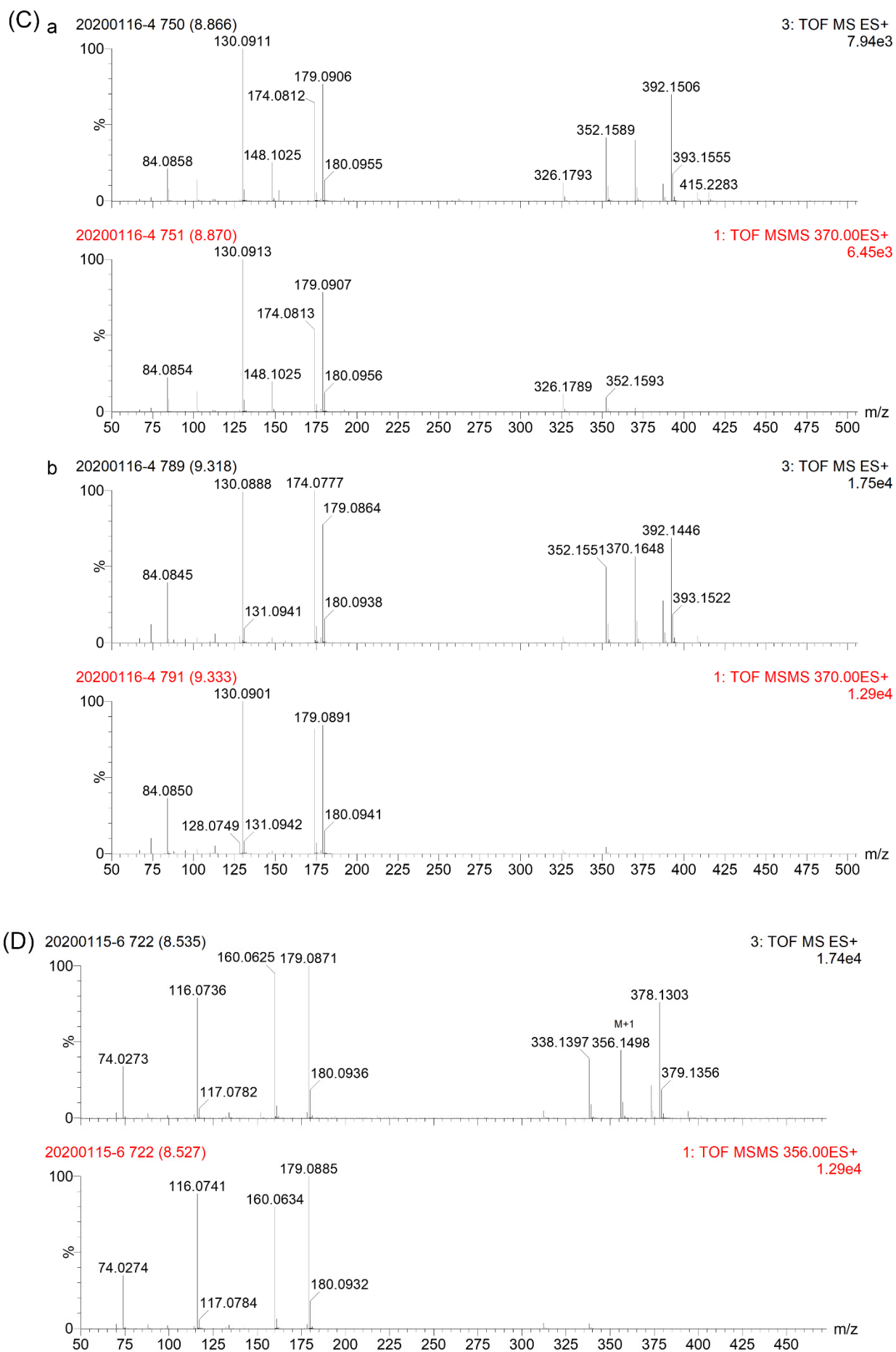


Figure S2. Effects of reaction conditions on IDO and LGOX activity. (A) Activity profiles of IDO and LGOX as functions of temperature; (B) activity profiles of IDO and LGOX as functions of pH and buffer; (C) IDO thermostability; (D) LGOX thermostability.

3. LC-MS analysis

LC-MS analysis of five products were recorded on the MALDI SYNAPT MS SYSTEM-WATERS instrument.





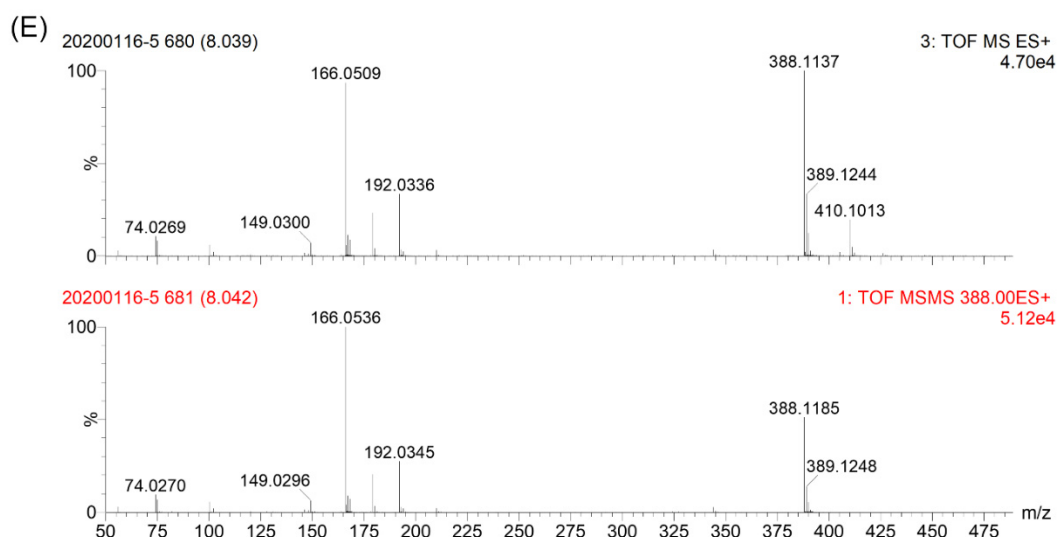
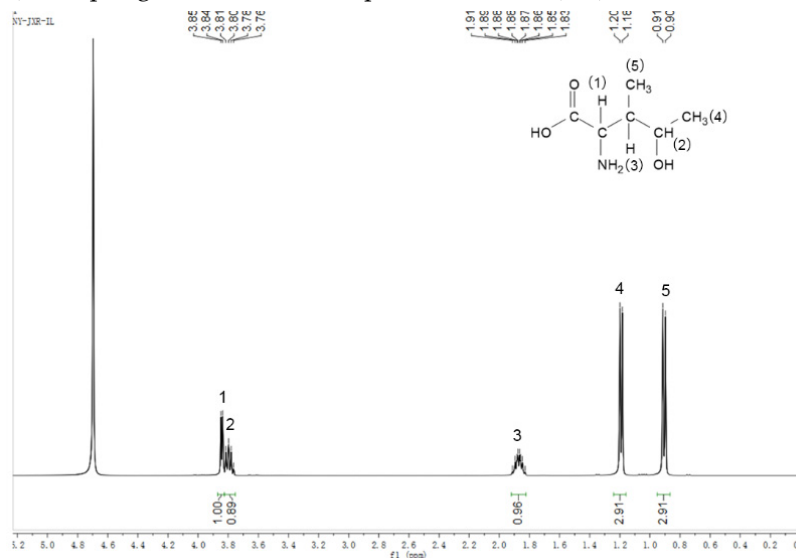


Figure S3. Products analysis by LC-MS. (A) Hydroxyl amino acids were generated from the two-step system with different L-amino acids as substrates, where the derivatives of five products by Fmoc-Cl were analyzed by LC-MS; (B) mass spectrometry analysis of the derivative of 4-hydroxy-L-leucine by Fmoc-Cl; (C) mass spectrometry analyses of the derivatives of 5-hydroxy-L-norleucine (a) and 4-hydroxy-L-norleucine (b) by Fmoc-Cl; (D) mass spectrometry analysis of the derivative of 4-hydroxy-L-norvaline by Fmoc-Cl; (E) mass spectrometry analysis of the derivative of L-methionine sulfoxide by Fmoc-Cl.

4. Nuclear magnetic resonance (NMR) analysis of (2S,3R,4S)-4-hydroxyisoleucine (4-HIL)

^1H NMR of 4-HIL was recorded on Avance 400 (Bruker, Billerica, MA, USA) at 400 MHz (^1H NMR). Chemical shifts were reported in ppm down field from internal Me_4Si . Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), br (broad). Coupling constants were reported in Hertz (Hz).



^1H NMR (400 MHz, D_2O) δ 3.84 (d, J = 4.4 Hz, 1H), 3.79 (dd, J = 13.6, 6.8 Hz, 1H), 1.92 – 1.82 (m, 1H), 1.19 (d, J = 6.3 Hz, 3H), 0.91 (d, J = 7.1 Hz, 3H).

Figure S4. Analysis of 4-HIL generated from L-isoleucine hydroxylation by ^1H -NMR spectrum.



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