Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Not exclusively the activity, but the sweet spot: a dehydrogenase point mutation synergistically boosts activity, substrate tolerance, thermo-stability and yield

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SUPPORTING INFORMATION

Contents:

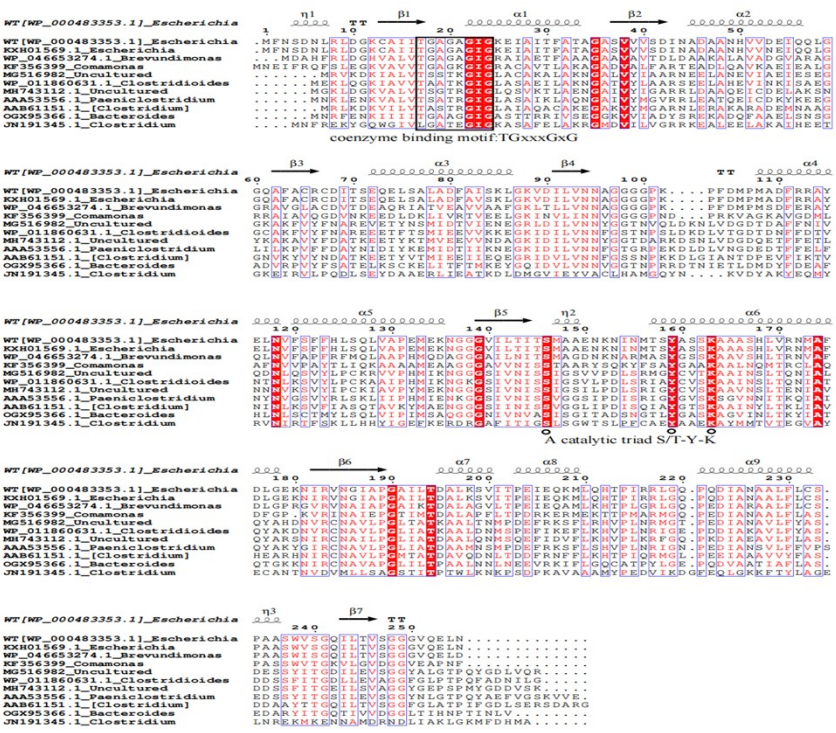

Fig.S1 Alignment of the wild-type sequence with the 7α-HSDH sequence reported in the literatures. The identical and similar residues are indicated with red shading and boxes, respectively. Wavy lines and arrows represent the α-helix and β-strand, respectively. The 7α-HSDH sequences from the top to the bottom rows are with accession numbers WP000483353.1, KXH01569.1, WP046653274.1, KF356399, MG516982, WP_011860631.1, MH743112.1, AAA53556.1, AAB61151.1, OGX95366.1, JN191345.1, as indicated.

The wild-type sequence:

MFNSDNLRLDGKCAIITGAGAGIGKEIAITFATAGASVVVSDINADAANHVVD
EIQQLGGQAFACRCDITSEQELSALADFAISKLGKVDILVNNAGGGGPKPFDM
PMADFRRAYELNVFSFFHLSQLVAPEMEKNGGGVILTITSMAAENKNINMTS
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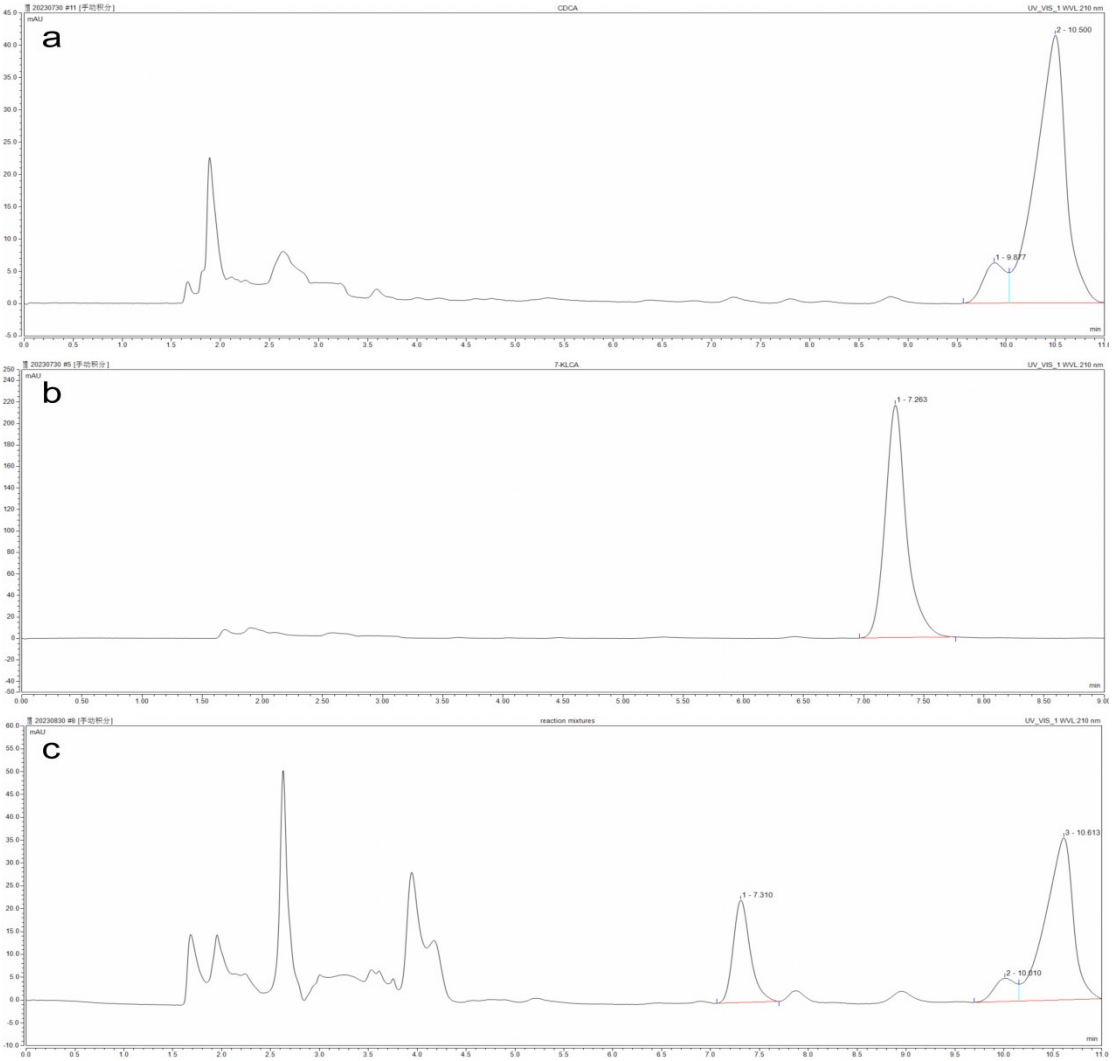

Fig.S2 Chromatograms of (**a**) standard CDCA samples, (**b**) standard 7-KLCA samples and (**c**) reaction mixtures. The peak between the retention time points 10-11 min represents the CDCA sample. The peak between the retention time points 7-7.5 min represents the 7-KLCA sample. Y axis: intensity (counts); X axis: retention time (min). HPLC (Thermo SCIENTIFIC, DIONEX UltiMate 3000) analysis was performed under a 60% acetonitrile mobile phase at a flow velocity of 1.0 mL/min, equipped with a C18 column (250 mm × 4.6 mm, 5 μm, WelChrom, Shanghai) maintained at 30° C, and detected at wavelength of 285 nm (Thermo SCIENTIFIC,

DAD-3000).

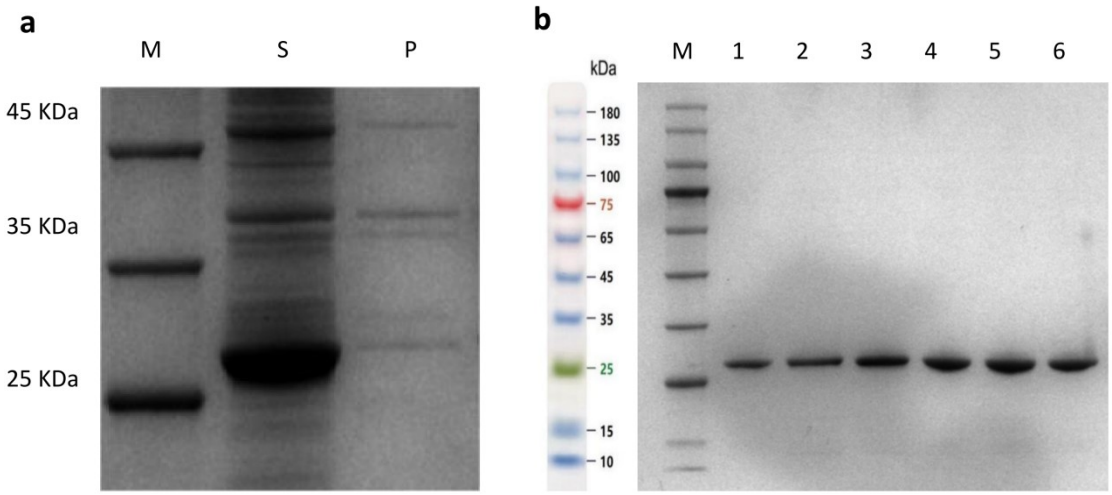

Fig.S3 SDS-PAGE analysis of soluble expression and purification of enzyme. (**a**) The soluble expression profile of 10 g/L WT enzymes, where 'M' denotes the marker, 'S' represents the supernatant, and 'P' indicates the precipitate. (**b**) The SDS-PAGE of the purified enzymes. 'M': the marker. Lane 1: WT, lane 2: I192T, lane 3: I201M, lane 4: I205S, lane 5: I192T/I205S, and lane 6: I192T/I201M/I205S. The concentration of each purified protein added is 0.1 mg/mL.

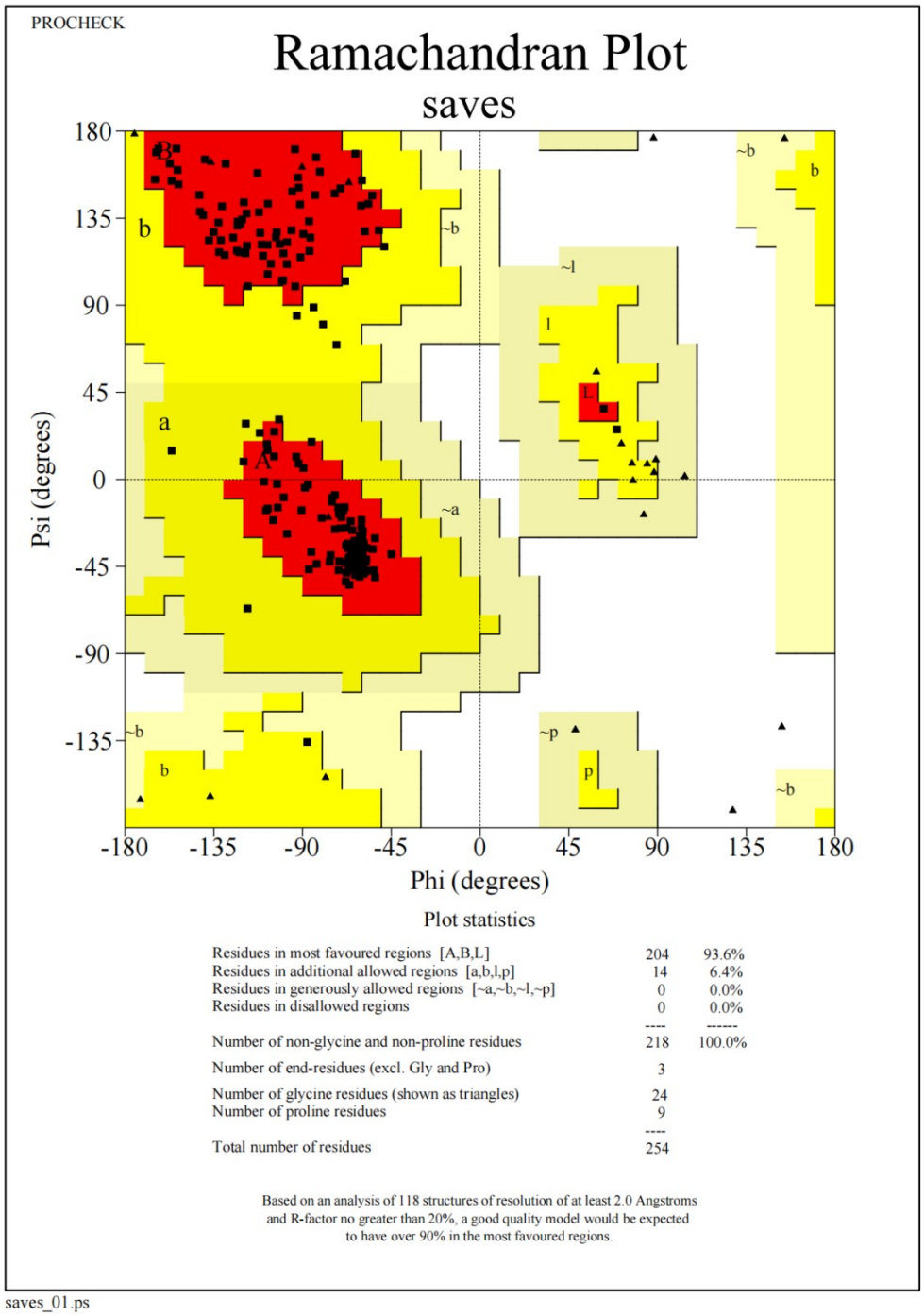

Fig.S4 The Ramachandran plot result.

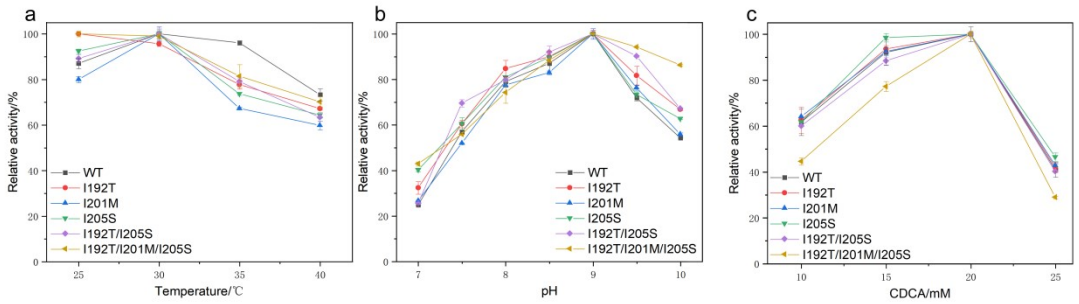

Fig.S5 The impact of temperature, pH and substrate concentration to the enzyme activity. (**a**) optimal temperature, (**b**) optimal pH, and (**c**) optimal substrate concentration for both the WT and mutants. The reaction conditions were in consistent with those used for enzyme activity assays. Activity was measured in 50 mM potassium phosphate buffer (pH 7.0-8.0), Tris-HCl buffer (pH 8.5-9.0) and glycine-NaOH buffer (pH 9.5-10.0).

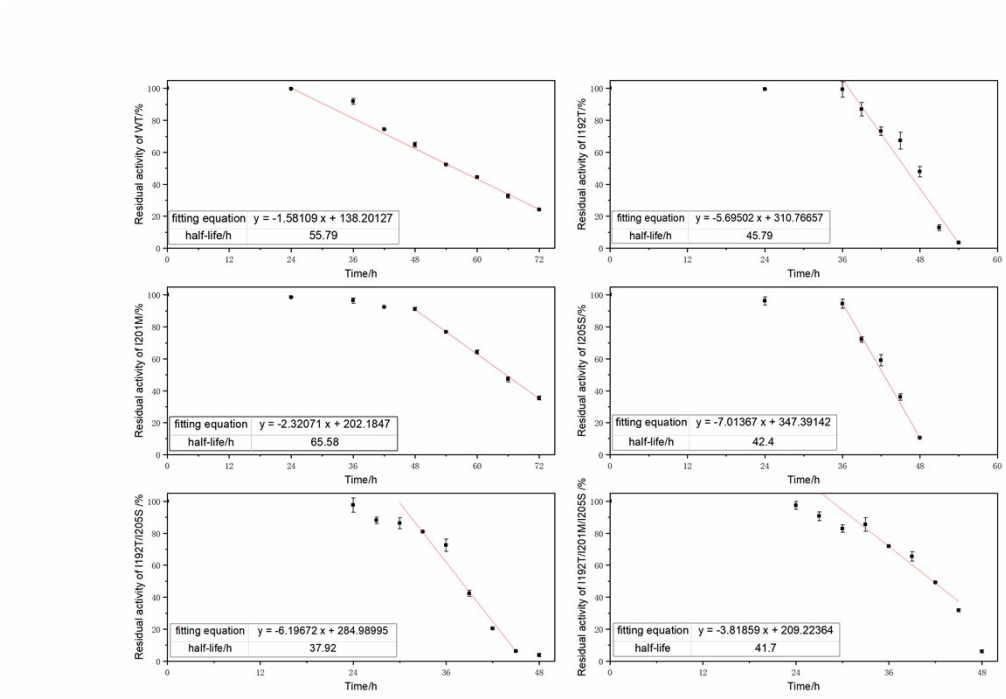

Fig.S6 The half-life of the WT and mutant enzymes.

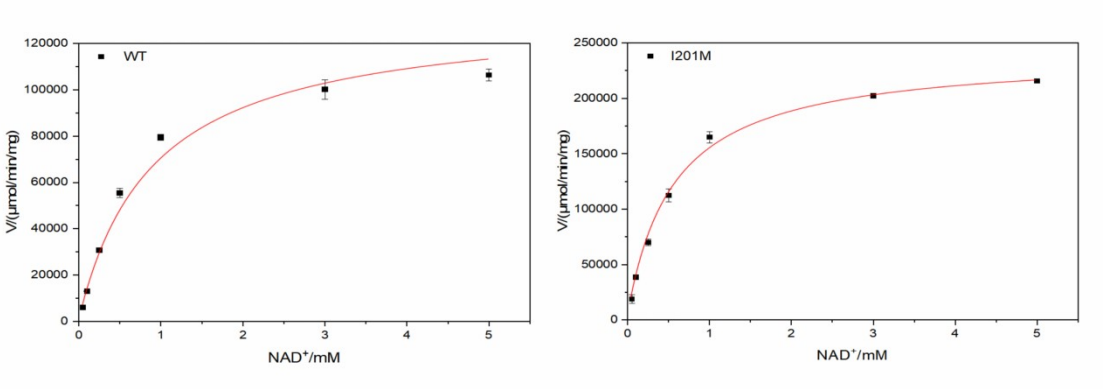

Fig.S7 The kinetic curves of the WT enzyme and the mutant I201M enzyme toward NAD^+. Assay conditions for kinetic resolution: 100 mM potassium phosphate buffer pH 7.5, 5 mM CDCA, 0.05-5 mM NAD^+, approximately 1 mg/mL purified enzyme (dilute 800 times), 30 °C. Y axis: activity normalized to μmol yield per minute reaction per mg purified enzyme.