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## **Electronic Supplementary Information**

## Rational engineering of Acinetobacter tandoii glutamate dehydrogenase for

## asymmetric synthesis of L-homoalanine through biocatalytic cascades

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Enzyme	Activity (U/mg)
Acinetobacter tandoii GluDH	57.3 ± 9.1
Cupriavidus necator GluDH	42.5 ± 3.7
Geobacillus thermocatenulatus GluDH	31.4 ± 1.2

Table S1. Enzyme activities of dual cofactor dependent GluDHs towards 2-ketobutyric acid in laboratory.

Table S2. Kinetic paramrters of *At*GluDH towards NADH and NADPH.

	$k_{cat}$ (s <sup>-1</sup> )	K <sub>m</sub> (mM)	k <sub>cat</sub> / K <sub>m</sub> (mM <sup>-1</sup> s <sup>-1</sup> )
NADH <sup>a</sup>	3673.1±121.9	0.52±0.08	7063.65
NADPH <sup>b</sup>	4013.8±208.5	3.87±0.14	1037.16

<sup>a</sup> Data for NADH and NADPH was measured with 200 mM  $NH_4Cl/NH_4OH$  buffer (pH 9.5), 50 mM 2-ketobutyric acid, 0.01-5 mM NADH or 0.01-30 mM NADPH and at 30°C.

Primer	Sequence (5'-3')
K76G-F	GGT <b>GGC</b> GGCGGTATTCGTTACCAT
K76G-R	TGGTCCACGCGACAAATTATGTTG
K76A-F	GGT <b>GCG</b> GGCGGTATTCGTTACCAT
K76A-R	TGGTCCACGCGACAAATTATGTTG
K76V-F	GGT <b>GTG</b> GGCGGTATTCGTTACCAT
K76V-R	TGGTCCACGCGACAAATTATGTTG
K76L-F	GGT <b>CTG</b> GGCGGTATTCGTTACCAT
K76L-R	TGGTCCACGCGACAAATTATGTTG
K76I-F	GGT <b>ATC</b> GGCGGTATTCGTTACCAT
K76I-R	TGGTCCACGCGACAAATTATGTTG
S355A-F	GTG <b>GCT</b> TACTTCGAGTGGGTTCAA
S355A-R	GGTTACACCGCCAGCATTACAGAG
S355V-F	GTG <b>GTG</b> TACTTCGAGTGGGTTCAA
S355V-R	GGTTACACCGCCAGCATTACAGAG
T180A-F	GTG <b>GCG</b> GGTAAACCTGTACATTTAGGT
T180A-R	TACACCTGTGACAGTATGACCCTT
T180V-F	GTG <b>GTG</b> GGTAAACCTGTACATTTAGGT
T180V-R	TACACCTGTGACAGTATGACCCTT
T180S-F	GTG <b>TCT</b> GGTAAACCTGTACATTTAGGT
T180S-R	TACACCTGTGACAGTATGACCCTT
T180C-F	GTG <b>TGC</b> GGTAAACCTGTACATTTAGGT
T180C-R	TACACCTGTGACAGTATGACCCTT

Table S3. Primers used for site-directed mutagenesis of AtGluDH.

Energy (kcal/mol) <sup>a</sup>	AtGluDH-WT	AtGluDH-K76L/T180C
VDW	-11.32±2.83	-14.18±2.97
EEL	-32.49±8.33	-10.71±8.46
GB	13.51±7.21	-5.77±7.14
GBSURF	-2.99±0.07	-3.09±0.005
GBGAS	-43.81±8.09	-24.90±7.90
GBSOLV	10.52±7.21	-8.87±7.13
GBTOT	-33.30±3.06	-33.76±3.76
PB	-20.64±9.44	-44.51±5.15
PBNPOLAR	-13.62±0.24	-13.74±0.15
PBDISPER	20.52±0.62	20.71±0.62
PBGAS	-43.81±8.09	-24.90±7.90
PBSOLV	-13.75±9.73	-37.54±5.21
РВТОТ	-57.56±10.16	-62.44±6.81

Table S4. Binding free energy analysis using the Molecular Mechanics Poisson Boltzmann (Generalized Born) surface area method.

<sup>a</sup> VDW: van der Waals energy. EEL: energy of electrostatic. GBTOT/PBTOT: final binding free energy.



Figure S1. (A) Enzyme inactivation assay at different temperatures for 12 h. (B) Time courses of thermal inactivation at 50°C. Purified *At*GluDH-WT was incubated at a certain temperature for a set time, and then added into the reaction mixure. A 200  $\mu$ L-scale reaction mixture containing a certain amount of purified *At*GluDH-WT, 2-ketobutyric acid (50 mM), 0.1 mM NADH, 200 mM NH<sub>4</sub>Cl/NH<sub>4</sub>OH buffer (pH 9.5). Then, the activity was measured by monitoring the absorbance at 340 nm ( $\epsilon$ =6220 M<sup>-1</sup> cm<sup>-1</sup>) at 30°C.



Figure S2. Reaction curve at different pH. A 20 mL-scale reaction mixture contained *Ec*TD (3 g/L), *At*GluDH-WT (10 g/L) and *Bm*GDH (3 g/L) which were prepared as lyophilized cells, 0.5 M L-threonine, 0.1 mM NADH, 0.6 M glucose, 200mM (NH<sub>4</sub>)<sub>3</sub>PO<sub>4</sub> buffer (pH 8.0-8.5) or 200 mM NH<sub>4</sub>Cl/NH<sub>4</sub>OH buffer (pH 9.0-10.0), and at 30°C.



Figure S3. Cascade reaction curves with 1 M L-threonine as substrate. A 20 mL-scale reaction mixture contained *Ec*TD (3 g/L), *At*GluDH-WT (10 g/L) and *Bm*GDH (3 g/L) which were prepared as lyophilized cells, 1 M L-threonine, 0.1 mM NADH, 1.2 M glucose, 200 mM  $NH_4Cl/NH_4OH$  buffer (pH 9.5), and at 30°C. Error bars represent the standard deviation of three independent experiments.



Figure S4. The conversion of the reductive amination of 2-ketobutyric acid by wild type *At*GluDH. A 1 mL-scale reaction mixture contained *At*GluDH-WT (10 g/L) and *Bm*GDH (3 g/L) which were prepared as lyophilized cells, 2-ketobutyric acid (200-1000 mM), 0.1 mM NADH, glucose (1.2 x substate concentration), 200 mM  $NH_4Cl/NH_4OH$  buffer (pH 9.5), and at 30°C within 24 h. Error bars represent the standard deviation of three independent experiments.



Figure S5. Homology model of AtGluDH with NAD<sup>+</sup>,  $\alpha$ -ketoglutarate and 2-ketobutyric acid. NAD<sup>+</sup>,  $\alpha$ -ketoglutarate and 2-ketobutyric acid are shown as sticks.

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AtGluDH-A.tandoii LfLeuDH-L.fusiformis CnLeuDH-C.necator PpGluDH-P.putida EcGluDH-E.coli CgGluDH-C.glutanicum PeGluDH-P.entomophila LsGluDH-T.sphaericus GsGluDH-G.stearothermophilus BsGluDH-B.subtilis BmGluDH-B.megaterium CsGluDH-C.symbiosum	26 19 41 46 45 43 54 30 31 33 43	YLEEDLIPFINTIKRPKRALIVDVPIVNDDGSIQHFEGYRVQHNLSRGEG 	GGIRYHPDV GGARMWTYA GGGVRFHDDV GGLRFHPSV GGLRFHPSV GGLRFHPSV GGLRFHPSV GGVRFHPDV GGGIRFHPDV GGGIRFHPDV GGGIRFHPNV GGGIRFAPSV
AtGluDH-A.tandoii LfLeuDH-L.fusiformis CnLeuDH-C.necator PpGluDH-P.putida EcGluDH-E.coli CgGluDH-C.glutamicum PeGluDH-P.entomophila LsGluDH-L.sphaericus GsGluDH-G.stearothermophilus BsGluDH-B.subtilis BmGluDH-B.megaterium CsGluDH-C.symbiosum	86 50 100 103 102 102 100 111 89 90 92 100	ELNEVMAN SAWMTIKTAVLNLPYGGAKGGIRVDPRKLSPRELERLT SEENAIEDALRHARGMTYKNAAACLNLGGGKIVIIGDPFKDKNEMFRAL TLSEVMAN SAWMSYKNAAAVNYPYGGAKGGIRVDPRTLSHAELERLT NLSVLKFNGFEQVFKNALTILPMGGGKGGSDFDPKGKSDAPVMRFC NLSILKFNGFEQFFKNALTILPMGGGKGGSDFDPKGKSDAPVMRFC NLGVKFNGFEQFFKNALTICPMGGGKGGSDFDPKGKSDAPVMRFC NLGVLKFNGFEQFFKNALTSLPMGGGKGGSDFDPKGKSDAPVMRFC NLGVLKFNGFEQFFKNALTSLPMGGGKGGSDFDPKGKSDAPVMRFC NLGVLKFNGFEQFFKNALTSLPMGGGKGGSDFDPKGKSDAPVMRFC NLGVLKFNGFEQFFKNALTSLPMGGGKGGSNFDPKGKSDAPVMRFC NLGVLKFNGFEQFFKNALTSLPMGGGKGGSNFDPKGKSDAPVMRFC NLGVLKFNGFEQFFKNALTGOPIGGGKGGIVCDPRKKSDAPVMRFC NLGVLKFNGFEQFFKNALTGOPIGGGKGGIVCDPRKKSDAPVMRFC NLGVLKFNGFEQFFKNALTGOPIGGGKGGIVCDPRKKSDAPVMRFC NLSIMFNGFEQFKNSLKCGIVDLPYGGGKGGIVCDPRKMSFCFLERLSI TEKEVKALSIWMSLKCGIVDLPYGGGKGGIVCDPRNMSFCFLERLSI NLSIMKFNGFEQFKDSLTTLPMGGAKGSDFDVMKSDSUPWRFC	RRFTTEISPI GRFIQGLNG. RRYTSEINIG. QAFMSELYRH QALMTELYRH QAFMTELYRH RGYVRAISQI RGYVRAISQI QAFMTELYRH
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Figure S6. Multiple-sequence alignment of AADHs from some different sources with the help of T-coffee and Espript 3.



Figure S7. (A and B) Relative activity of the mutants of AtGluDH compared to the wild-type protein. Reaction conditions: 200 µL final volume, 200 mM NH<sub>4</sub>Cl/NH<sub>4</sub>OH buffer (pH 9.5), 0.1 mM NADH, 50 mM 2-ketobutyric acid and at 30°C. Error bars represent the standard deviation of three independent experiments.



Figure S8. (A) Distance analysis between the reactive carbonyl carbon (C2) of the substrate and the hydride donating/accepting carbon (C4) of the nicotinamide group of the coenzyme in MD simulations. (B) RMSD analysis results.



Figure S9. (A) SDS-PAGE of *At*GluDH and mutants. L: cell free extract, N: precipitation, P: pure protein elution fraction, M: marker. (B) SDS-PAGE of the co-expression systems. Co1: *E.coli* cells co-expressing *At*GluDH (K76L/T180C) and *Bm*GDH, Co2: *E.coli* cells co-expressing *At*GluDH (K76L/T180C), *Bm*GDH and *Ec*TD, L: cell free extract, N: precipitation, M: marker. Protein molecular weight: *At*GluDH (46.7 kDa), *Bm*GDH (28.2 kDa), *Ec*TD (56.2 kDa).



Figure S10. (A) HPLC chromatogram of racemic 2-aminobutyric acid. (B) HPLC chromatogram of L-2-aminobutyric acid. (C) HPLC chromatogram of product L-2-aminobutyric acid yielded by *At*GluDH.

Figure S11. NMR spectra of the purified L-homoalanine. (A) <sup>1</sup>H NMR; (B) <sup>13</sup>C NMR. <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O):  $\delta$  = 3.71 (td, *J* = 5.8, 2.8 Hz, 1H), 1.89 (m, 2H), 0.97 (td, *J* = 7.6, 1.7 Hz, 3H); <sup>13</sup>C NMR (150 MHz, D<sub>2</sub>O):  $\delta$  = 174.9, 55.8, 23.7, 8.5.