Catalysis Science & Technology

Supplementary information

Table S1. The RMSD of enzyme backbone between each representative conformation

RMSD of enzyme backbone	WT/pH 5.0	WT/pH 7.0	E88R/pH 5.0	E88R/pH 7.0
WT/pH 5.0	0	1.332	1.465	1.823
WT/pH 7.0	1.332	0	1.169	1.470
E88R/pH 5.0	1.465	1.169	0	1.720
E88R/pH 7.0	1.823	1.470	1.720	0

Table S2. Primers used for the construction of variants

Primes	Sequences (5'-3')
E81R-F	GGAGGCCTGGCAAAGATCCGCGGCAT
E81R-R	CCCTTCTATGCCGCGGATCTTTGCCAG
E88R-F	GGCATAGAAGGGCGCTTCCGCCGCTAT
E88R-R	GGCAATATAGCGGCGGAAGCGCCCTTC
E93R-F	TTCGAACGCTATATTGCCCGCAACCGC
E93R-R	CTGATCGCGGTTGCGGGCAATATAGCG
D96R-F	TTCGAACGCTATATTGCCGAAAACCGC
D96R-R	CACGCCTTCCTGGCGGCGGTTTTCGGC
D159R-F	ATCCGTGAAATGGGGGCCCGCGCAATT
D159R-R	GGACTCGCTGGGAATTGCGCGGGCCCC
S163R-F	ATCCGTGAAATGGGGGCCGATGCAATT
S163R-R	CAGGTTCACGGACTCGCGGGGAATTGC
E164R-F	ATCCGTGAAATGGGGGCCGATGCAATT
E164R-R	CAGGTTCACGGAGCGGCTGGGAATTGC
E264R-F	TCGGTAAAGATGGACCGCCGCAGCCTG
E264R-R	CCATCGGTAAAGATGGACCGCCGCAGC
E268R-F	CCATCGGTAAAGATGGACGAGCGCAGC
E268R-R	CTTGCGCACACGGCGCAGGCTGCGCTC
E344R-F	TTCGACTTGGCATTGGGCAGGCTGCCT
E344R-R	TGTTTTCTCGGAGCGAGGCAGCCTGCC
S345R-F	TTCGACTTGGCATTGGGCAGGCTGCCT
S345R-R	TGTTTTCTCGCGCTCAGGCAGCCTGCC

E346R-F	TTCGACTTGGCATTGGGCAGGCTGCCT
E346R-R	CGCTGTTTTGCGGGACTCAGGCAGCCT
T348R-F	TGGGCAGGCTGCCTGAGTCCGAGAAA
T348R-R	ATCATCGAGCGCGCGTTTCTCGGACTC
D352R-F	CTGCCTGAGTCCGAGAAAACAGCGCTC
D352R-R	ACGATAGCGATCGCGGAGCGCTGTTTT
S363R-F	TATCGTTCACTGCTACCCGATGTGCGT
S363R-R	TAGGAATTTCAAGCGACGCACATCGGG
S405R-F	TTCTCGTTGTTTGCGCTCATGGACGAG
S405R-R	CTTGTACTGGTCGCGCTCGTCCATGAG
D448R-F	GATTACTACACCTTGATTGACCTGCAG
D448R-R	CTTCGACGTCACGCGCTGCAGGTCAAT
E456R-F	CAGGACGTGACGTCGAAGCTTTATGGC
E456R-R	TTTCGAGAACGCGCGGCCATAAAGCTT
D520R-F	ATAGGTCGTGCGCTGCGACAAATGGCT
D520R-R	CGCGTACAGCTCGCGAGCCATTTGTCG
T527R-F	ATGGCTGACGAGCTGTACGCGCAATAC
T527R-R	GTTCCCTTGCTGGCGGTATTGCGCGTA

Table S3. The protonation state of residues predicted by PDB2PQR

Enzyme (condition)	Protonation state	Residue	Enzyme (condition)	Protonation state	Residue
WT (pH 7.0)	ASPP	D287	E88R (pH 7.0)	ASPP	D287
	HSE	H123		HSE	H123
		H381			H381
	HSD	Other histidine		HSD	Other histidine
WT (pH 5.0)	ASPP	D286	E88R (pH 5.0)	ASPP	D286
		D287			D287
		D336			D336
		D445			D445
	GLUP	E84		GLUP	E84
		E124			E124
		E155			E155
		E220			E220
		E420			E420
	HSP	H123		HSP	H123
		H277			H277
		H330			H330
		H381			H381
		H410			H410
	HSD	Other histidine		HSD	Other histidine

Note:

ASPP: Protonated aspartic acid, proton on OD2;

GLUP: Protonated glutamic acid, proton on OE2;

HSP: Protonated histidine;

HSD: Neutral histidine, proton on ND1;

HSE: Neutral histidine, proton on NE2.

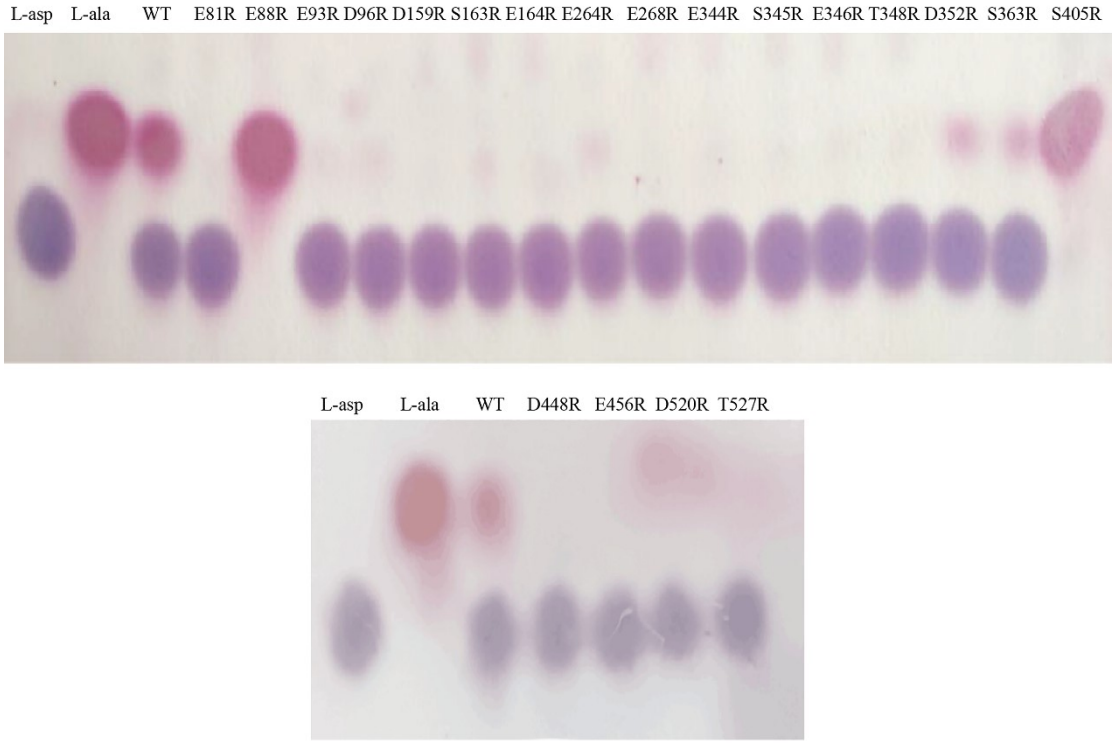

Fig. S1. Thin-plate chromatography analysis for screening of variants. The reaction was carried out at 37°C and 200 rpm for 4 h, supplying 100 mM L-aspartic acid and the cell biomass of OD_{600}=2.5. Samples of L-aspartic acid and L-alanine were spotted as controls and indicated by L-asp and L-ala, respectively. WT: wild-type ASD.

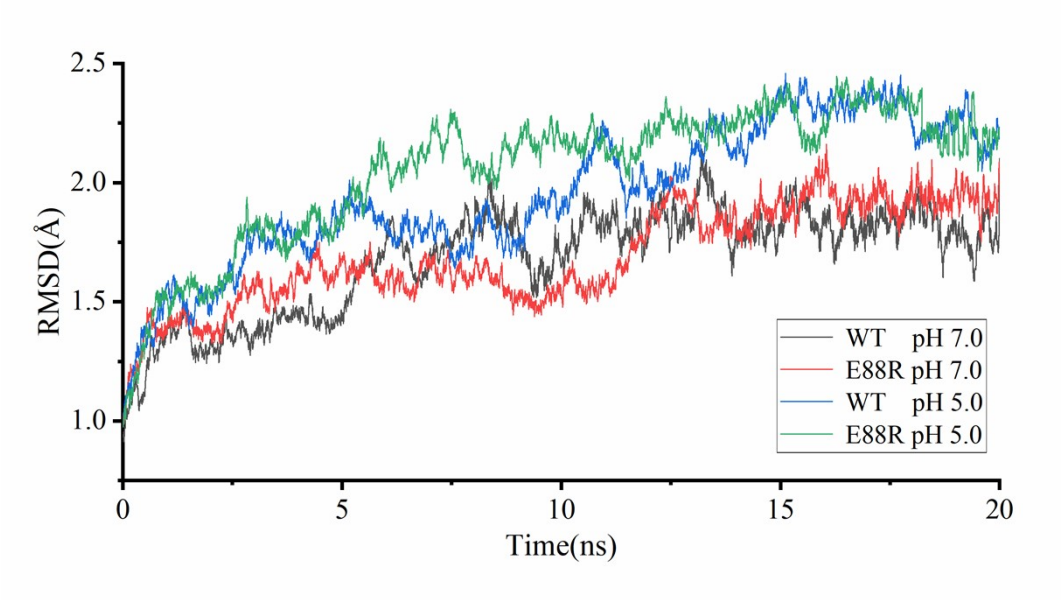

Fig. S2. The RMSD of the wild-type ASD (WT) and the E88R variant analyzed under acidic and neutral conditions.

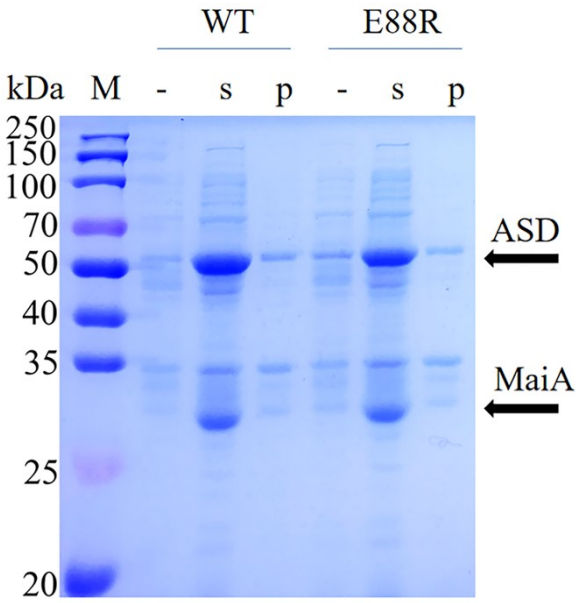

Fig. S3. Expression level of recombinant enzymes in the host strain were analyzed by SDS-PAGE. M: standard marker proteins, -: control, S: supernatants of cell lysates, P: precipitate of cell lysates. Recombinant maleate isomerase (MaiA) and ASD are indicated by arrows.