An extended bacterial reductive pyrimidine degradation pathway that enables nitrogen release from β-alanine

Jinyu Yin[1†], Yifeng Wei[2†], Dazhi Liu[1], Yiling Hu[1], Qiang Lu[1], Ee Lui Ang[2], Huimin Zhao[2, 3*], Yan Zhang[1*]

List of materials in Supporting Information

Supplementary methods

Supporting Figures (Fig. S1-11)

Supporting Tables (Table S1-3)

SUPPLEMENTARY METHODS

Preparation of defined medium for *B. smithii*

To prepare defined medium, 9 g Na2HPO4·12H2O, 1.5 g KH2PO4, 0.01 g MnSO4·H2O, 0.2 g MgSO4·7H2O, 0.01 g CaCl2·2H2O, 5 mg Na2·EDTA·2H2O, 1.5 mg CoCl2·6H2O, 1 mg ZnCl2, 0.1 mg H3BO3, 0.2 mg CuCl2·2H2O, 0.1 mg Na2MoO4·2H2O, 0.2 mg NiSO4·6H2O, 1 mg FeSO4·7H2O, 0.2 mg Na2SeO3, 0.4 mg Na2WO4·2H2O, 20 μg biotin, 20 μg folic acid, 100 μg pyridoxine-HCl, 50 μg thiamine-HCl, 50 μg riboflavin, 50 μg nicotinic acid, 50 μg D-Ca-pantothenate, 1 μg vitamin B12, 50 μg p-aminobenzoic acid, 50 μg lipoic acid, 17.5 μg inositol, 3.75 μg choline, 1.5 g Na-pyruvate and 20 g glucose were dissolved in 1 L distilled water, NH4Cl, uracil, uridine or cytidine was added as the sole nitrogen source, adjusted to a molar equivalent of 20 mM nitrogen. The medium was then filtered through a sterile 0.22 μm filter.

Purification and activity assay of *Bs*ALD

The gene encoding *Bs*ALD that was used as a coupling enzyme for spectrophotometric assays was amplified by PCR from *Bacillus subtilis* genomic DNA, using the primer pair 3F/3R (Table S1). The PCR product was inserted into customized expression vectors (encoding an N-terminal MGSSHHHHHHSQSGSG tag) by Gibson assembly to form pET28-BsALD. The recombinant plasmid was confirmed by DNA sequencing through Tsingke (Beijing, China), and was transformed into BL21 (DE3) E. coli cells. Cell culture and protein induction were performed exactly the same as described for PydD/E. Cell paste from 2 L culture was resuspended in 100 mL lysis buffer containing 40 mM HEPES, pH 7.4, 500 mM NaCl. Cells were then lysed by French Press (Panda plus, Niro Soavi Co., Italy). After cell debris removal by centrifugation at 20,000 ×g, the cell lysate was filtered and loaded on a 10 mL Ni HisTrap HP column and washed with 4 CV of lysis buffer. The column was then eluted with a linear gradient from 0-300 mM imidazole by mixing lysis buffer and elution buffer containing 40 mM HEPES, pH 7.4, 500 mM NaCl, 500 mM imidazole. The *Bs*ALD protein was eluted roughly at 100 mM imidazole. The fractions containing *Bs*ALD were pooled together and dialyzed against 4 L dialysis buffer containing 40 mM HEPES, pH 7.0, 100 mM NaCl overnight. The typical yield of *Bs*ALD from 2 L cell culture was 190 mg. The protein was purified to high homogeneity, analysed by 12% SDS-PAGE. The specific activity of purified BsALD was measured under the PydD coupling assay conditions. A 200 μL of reaction mixture containing 100 mM HEPES buffer, pH 8.0, 5 mM NAD+, 20 mM L-alanine, and 200 ng *Bs*ALD was continuously monitored for the change of A340 using a plate reader (Tecan M200).

Table S1. Oligonucleotides used for cloning and sequencing

Oligos	Target	Sequence (5' to 3')	Application
1F	*pydD*	(5'-TGAAAACCTGTACTTCCAATCCAATAGTGATTATTTATCGATAAAAGAAC-3')	Construct HT-PydD
1R	*pydD*	(5'-TTCGGATCCGTTATCCACTTCCAATTTACACAGATAGCGTTTCTT-3')	Construct HT-PydD
2F	*pydE*	(5'-TGAAAACCTGTACTTCCAATCCAATGTGGAAGGATTTTATTACGAAC-3')	Construct HT-PydE
2R	*pydE*	(5'-TTCGGATCCGTTATCCACTTCCAATTTATGAAACTGCTGAAGATTTTG-3')	Construct HT-PydE
3F	*Bs*ALD	(5'-CCAGAGCGGATCAGGAATGATCATAGGGGTTCCTAAAGAG-3')	Construct pET-28a(+)-His$_6$-*Bs*ALD
3R	*Bs*ALD	(5'-CCAATTGAGATCTGCCATATGTTAAGCACCGCCACAGATGATTC-3')	Construct pET-28a(+)-His$_6$-*Bs*ALD

Table S2. Growth of *Bacillus smithii* in defined media containing pyrimidine as the sole nitrogen source

Bacterial strains Nitrogen source	*Bacillus smithii*	
	Growth	3-HP secreted
none	-	-
NH_4Cl	+	-
uracil	+	+
uridine	+	+
cytidine	+	-

Table S3. A list of bacteria with Pyd gene clusters containing PydABCD (and PydE)

Phylum	Class	Organism	PydA	PydB	PydC	PydD	PydE
Firmicutes	Bacilli	*A. pallidus*	A0A223E4N8	A0A223E4K1	A0A223E4I5	A0A223E4L3	A0A223E4H9
Firmicutes	Bacilli	*B. asahii*	A0A398B2G6	A0A398B195	A0A398B1I7	A0A398B0T7	A0A398B3Z0
Firmicutes	Bacilli	*B. coagulans*	A0A0C5CFU2	A0A0C5CCT3	A0A0C5C847	A0A0C5CFU6	A0A0C5CCT7
Firmicutes	Bacilli	*B. coagulans* 36D1	G2TR85	G2TR84	G2TR88	G2TR90	G2TR89
Firmicutes	Bacilli	*B. smithii*	A0A0H4P1N5	A0A0H4P5H6	A0A0H4PIJ5, A0A0H4PIK3	A0A0H4P1N9	A0A0H4P5H9
Firmicutes	Bacilli	*L. massiliensis* 4400831 = CIP 108448 = CCUG 49529	A0A0A3JZ82	A0A0A3J937	A0A0A3J5F8	A0A0A3JZ59	A0A0A3J5T1
Firmicutes	Bacilli	*R. sp.* TYF005	A0A3N6BSY1	A0A3N5ZTC8	A0A3N6ACG0	A0A3N5ZU34	A0A3N5ZTD4
Firmicutes	Clostridia	*C. algidixylanolyticum*	A0A419SSA0	A0A419SSE2	A0A419SSC9	A0A419SS74	A0A419SSG2
Firmicutes	Clostridia	[*Eubacterium*] *cellulosolvens* 6	I5AWA1	I5AWA0	I5AWA2	I5AW94	I5AW95
Firmicutes	Clostridia	*A. ruminis* DSM 5522	A0A1I0ZLN2	A0A1I0ZM62	A0A1I0ZLT7	A0A1I0ZLD1	A0A1I0ZLU6
Firmicutes	Clostridia	*A. ruminis*	A0A2G3E590	A0A2G3E5C8	A0A2G3E514	A0A2G3E5F6	A0A2G3E5D3
Firmicutes	Clostridia	*A. jejuensis* DSM 15929	A0A1M7AUR3	A0A1M7AV09	A0A1M7AV22	A0A1M7AVE2	A0A1M7AVG0
Firmicutes	Clostridia	*A. xylanovorans* DSM 12503	A0A1M7YA70	A0A1M7Y9Z9	A0A1M7YA28	A0A1M7YA39	A0A1M7YA15
Firmicutes	Clostridia	*A. mobilis* DSM 15930	A0A1M7F2L7	A0A1M7F211	A0A1M7F1X4	A0A1M7F341	A0A1M7F323
Firmicutes	Clostridia	*A.multivorans*	A0A239HS77	A0A239HRL2	A0A239HS20	A0A239HRK1	
Firmicutes	Clostridia	*B. sp.*	A0A350RPH5	A0A350RPH6	A0A350RPH4	A0A350RPI2	A0A350RPI1
Firmicutes	Clostridia	*B. sp.*	A0A3C0J5R4	A0A3C0J5N5	A0A3C0J6I7	A0A3C0J5L0	A0A3C0J5S7
Firmicutes	Clostridia	*C. lentocellum* (ATCC 49066/DSM 5427/NCIMB 11756/RHM5)	F2JM71	F2JM70	F2JM72	F2JM64	F2JM65
Firmicutes	Clostridia	*C. bacterium* BRH_c25	A0A101W7G8	A0A101W8C9	A0A101W6X6, A0A101W7F7	A0A101W790	
Firmicutes	Clostridia	*C. bacterium* GWB2_37_7	A0A1F8U3L1	A0A1F8U221	A0A1F8U2E7	A0A1F8U2P4	
Firmicutes	Clostridia	*C. acidisoli* DSM 12555	A0A1W1XNV9	A0A1W1XMX4	A0A1W1XNU6	A0A1W1XMP8	A0A1W1XMW9
Firmicutes	Clostridia	*C. autoethanogenum*	A0A3M0T022	A0A3M0SPC2	A0A3M0SPS6	A0A3M0SRE8	A0A3M0SQY0
Firmicutes	Clostridia	*C. autoethanogenum* DSM 10061	U5RVY0	U5RSI1	U5RWH7	U5RVY5	U5RSM4
Firmicutes	Clostridia	*C. beijerinckii* (*Clostridium MP*)	A0A1S8P8F7	A0A1S8QYR0	A0A1S8QYE7	A0A1S8P8M0	A0A1S8P8K9
Firmicutes	Clostridia	*C. beijerinckii* (*Clostridium MP*)	A0A1S8P7U8	A0A0B5QKE4	A0A1S8P7T5	A0A1S8P7T0	A0A1S8P839
Firmicutes	Clostridia	*C. beijerinckii* (*Clostridium MP*)	A0A1S8PFH4	A0A1S8Q8J0	A0A1S8Q8M2	A0A1S8PFN4	A0A1S8Q8G9
Firmicutes	Clostridia	*C. beijerinckii* (*Clostridium MP*)	A0A0B5QL98	A0A0B5QKE4	A0A0B5QPE4	A0A0B5QCQ0	A0A0B5QKD8
Firmicutes	Clostridia	*C. beijerinckii* (*Clostridium MP*)	A0A1W7LQQ8	A0A0B5QKE4	A0A1W7LM15	A0A1W7LTG9	A0A1W7LM10
Firmicutes	Clostridia	*C. beijerinckii* (*Clostridium MP*)	A0A1S9N1C6	A0A1S9N117	A0A1S9N0Z7	A0A1S8QRI1	A0A1S9N128
Firmicutes	Clostridia	*C. beijerinckii* (*Clostridium MP*)	A0A1B9BKP6	A0A0B5QKE4	A0A1B9BKS4	A0A1B9BKQ6	A0A1B9BKM4
Firmicutes	Clostridia	*C. beijerinckii* (*Clostridium MP*)	A0A1S8S4F4	A0A1S8S4S5	A0A1S8S4M0	A0A1S8S4D7	A0A1S8S4F1
Firmicutes	Clostridia	*C. beijerinckii* (ATCC 51743/NCIMB 8052) (*Clostridium acetobutylicum*)	A6LUT3	A6LUT2	A6LUT4	A6LUS6	A6LUS7
Firmicutes	Clostridia	*C. beijerinckii* NRRL B-598	A0A0K2MFY1	A0A0K2MFE3	A0A0K2MFP6	A0A0K2ML45	A0A0K2MFF1
Firmicutes	Clostridia	*C. carnis*	A0A3P6K848	A0A3P6KMN4	A0A3P6K857	A0A3P6JKC2	A0A3P6JH88
Firmicutes	Clostridia	*C. chromiireducens*	A0A399ILH0	A0A399IL93	A0A399ISF6	A0A399IL77	A0A399INK5
Firmicutes	Clostridia	*C. chromiireducens*	A0A1V4IHK3	A0A1V4IH40	A0A1V4IGU3	A0A1V4IGM3	A0A1V4IGU8
Firmicutes	Clostridia	*C. coskatii*	A0A166SRP5	A0A162L8S6	A0A166SRS4	A0A162JAQ4	A0A162LF43
Firmicutes	Clostridia	*C. diolis*	A0A2P8RIF7	A0A2P8RII9	A0A2P8RIF0	A0A2P8RIP8	A0A2P8RIM7
Firmicutes	Clostridia	*C. homopropionicum* DSM 5847	A0A0L6ZBA6	A0A0L6ZAV4	A0A0L6ZAU7, A0A0L6ZAU0	A0A0L6ZAT8	A0A0L6ZB99
Firmicutes	Clostridia	*C. hungatei*	A0A1V4SIN8	A0A1V4SGW4	A0A1V4SGV3, A0A1V4SGX9	A0A1V4SI22	A0A1V4SH37
Firmicutes	Clostridia	*C. intestinale* DSM 6191	A0A1M6AWV5	A0A1M6AWB9	A0A1M6AW27	A0A1M6AXG3	A0A1M6AXC4
Firmicutes	Clostridia	*C. intestinale* URNW	U2Q2J9	U2NPH7	U2NP64	U2NM35	U2NPU1
Firmicutes	Clostridia	*C. kluyveri*	A0A1L5F9I3	A0A1L5FA26	A0A1L5FE54	A0A1L5F9M1	A0A1L5F9N7

Firmicutes	Clostridia	*C. kluyveri* (ATCC 8527/DSM 555/NCIMB 10680)	A5MZW5	A5MZW7	A5MZW9	A5MZX0	A5MZX1
Firmicutes	Clostridia	*C. kluyveri* (NBRC 12016)	B9E3U1	B9E3U3	B9E3U5	B9E3U6	B9E3U7
Firmicutes	Clostridia	*C. ljungdahlii* (ATCC 55383/DSM 13528/PETC)	D8GT29	D8GT31	D8GT33	D8GT34	D8GT35
Firmicutes	Clostridia	*C. luticellarii*	A0A2T0BSD2	A0A2T0BSC0	A0A2T0BSH3	A0A2T0BSA8	A0A2T0BSB5
Firmicutes	Clostridia	*C. neonatale*	A0A2A7MJ06	A0A2A7MJQ1	A0A2A7MJ07	A0A2A7MIR0	A0A2A7MIN4
Firmicutes	Clostridia	*C. pasteurianum*	A0A1D9N1A6	A0A1D9N1C4	A0A1D9N1Q6	A0A1D9N156	A0A1D9N1M4
Firmicutes	Clostridia	*C. pasteurianum* BC1	R4K0I3	R4JZ11	R4K6E3	R4K0H9	R4JZ15
Firmicutes	Clostridia	*C. pasteurianum* (DSM 525/ATCC 6013)	A0A0H3J858	A0A0H3J065	A0A0H3J059	A0A0H3J856	A0A0H3J1E7
Firmicutes	Clostridia	*C. puniceum*	A0A1S8TH67	A0A1S8THH5	A0A1S8THC1	A0A1S8TH62	A0A1S8THH1
Firmicutes	Clostridia	*C. ragsdalei* P11	A0A1A6B1G0	A0A1A6B1N9	A0A1A6B1N1	A0A1A6B1G5	A0A1A6B1M2
Firmicutes	Clostridia	*C. saccharoperbutylacetonicum* N1-4(HMT)	M1MT42	M1N365	M1LXA7	M1MNW2	M1N370
Firmicutes	Clostridia	*C. scatologenes*	A0A0E3K0S0	A0A0E3GQW2	A0A0E3JYU1	A0A0E3M674	A0A0E3K0S2
Firmicutes	Clostridia	*C. sp.* (SY8519)	F7V837	F7V838	F7V836	F7V843	F7V834
Firmicutes	Clostridia	*C. sp.* BL-8	A0A1S8TS36	A0A1S8TRW3	A0A1S8TT21	A0A1S8TRZ1	A0A1S8TRW7
Firmicutes	Clostridia	*C. sp.* DL-VIII	G7M3S7	G7M3S8	G7M3S6	G7M3T4	G7M3T3
Firmicutes	Clostridia	*C. sp.* Maddingley MBC34-26	K6U657	K6U511	K6T9U8	K6TVC6	K6U508
Firmicutes	Clostridia	*C. tyrobutyricum* DIVETGP	W6N4J3	W6N3Q1	W6N4Z4	W6N4J7	W6NGD8
Firmicutes	Clostridia	*C. vincentii*	A0A2T0BKU7	A0A2T0BKR0	A0A2T0BKS2	A0A2T0BKS3	A0A2T0BKY4
Firmicutes	Clostridia	*D. sp.* OT	G2FWP0	G2FWP1	G2FWP2, G2FWP3	G2FWP5	G2FWP4
Firmicutes	Clostridia	*E. harbinense*	A0A386XE34	A0A386XPY5	A0A386XCP6	A0A386XIZ9	A0A386XLW0
Firmicutes	Clostridia	*E. harbinense* (DSM 18485/JCM 12961/CGMCC 1.5033/YUAN-3)	E6U518	E6U516	E6U520	E6U519	E6U509
Firmicutes	Clostridia	*E. oxidoreducens*	A0A1G6CKW4	A0A1G6CKX4	A0A1G6CKW1	A0A1G6CL41	A0A1G6CL40
Firmicutes	Clostridia	*H. salsuginis*	A0A1I4HJJ3	A0A1I4HH73	A0A1I4HIL3	A0A1I4HJD4	A0A1I4HJZ2
Firmicutes	Clostridia	*L. bacterium*	A0A3B9B4S5	A0A3B9B4Q5	A0A3B9B4U5	A0A3B9B4Q7	
Firmicutes	Clostridia	*L. bacterium* KHCPX20	A0A1H2QXM1	A0A1H2QXA4	A0A1H2QXK8	A0A1H2QWR9	A0A1H2QX07
Firmicutes	Clostridia	*L. bacterium* NLAE-zl-G231	A0A1I2XR06	A0A1I2XR46	A0A1I2XRG4	A0A1I2XTD4	A0A1I2XSB6
Firmicutes	Clostridia	*L. bacterium* TF09-5	A0A3A6HG77	A0A3A6HNT8	A0A3A6HQP2	A0A3A6HG24	A0A3A6HN59
Firmicutes	Clostridia	*N. peptidivorans*	A0A1H9ZZV8	A0A1I0A0Z5	A0A1H9ZZH7	A0A1I0A155	
Firmicutes	Clostridia	*O. metallireducens*	A0A285I060	A0A285I037	A0A285I027	A0A285I016	A0A285I2P4
Firmicutes	Clostridia	*P. cinnamivorans* DSM 12816	A0A1W2AUS7	A0A1W2AVT6	A0A1W2AVN4, A0A1W2AUP8	A0A1W2AUN2	A0A1W2AUK9
Firmicutes	Clostridia	*R. sp.* 499	A0A1Q9JEC1	A0A1Q9JEK9	A0A1Q9JED0	A0A1Q9JEH1	A0A1Q9JEA4
Firmicutes	Clostridia	*R. bacterium*	A0A3D5NG72	A0A3D5NFB9	A0A3D5NGG0	A0A3D5NFF9	A0A3D5NFD8
Firmicutes	Clostridia	*R. bacterium*	A0A3D4L3G8	A0A3D4L3G7	A0A3D4L584	A0A3D4L3W7	A0A3D4L4G8
Firmicutes	Clostridia	*R. bacterium*	A0A3D1HDR8	A0A3D1HEB7	A0A3D1HDM9	A0A3D1HDY4	A0A3D1HDZ6
Firmicutes	Clostridia	*R. bromii*	A0A414E3C8	A0A374NG71	A0A414E3D0	A0A414E3C5	A0A414E3D1
Firmicutes	Clostridia	*R. bromii*	A0A2N0UPM2	A0A2N0UPJ7	A0A2N0UPR2	A0A2N0UPN0	A0A2N0UPK9
Firmicutes	Clostridia	*R. bromii*	A0A1G5GLT1	A0A1G5GMB3	A0A1G5GLV9	A0A1G5GLG7	A0A1G5GMB9
Firmicutes	Clostridia	*R. bromii*	A0A374N5X1	A0A374NG71	A0A374NEW5	A0A374N5Y0	A0A374NEV0
Firmicutes	Clostridia	*R. bromii*	A0A2N0UI48	A0A2N0UHZ5	A0A2N0UHZ0	A0A2N0UI01	A0A2N0UI03
Firmicutes	Clostridia	*R. bromii*	A0A412DXP0	A0A412DXL5	A0A374NEW5	A0A412DXM2	A0A412DXJ0
Firmicutes	Clostridia	*R. champanellensis* (DSM 18848/JCM 17042/KCTC 15320/18P13)	D4L9N2	D4L9N1	D4L9N6	D4L9N4	D4L9N7
Firmicutes	Clostridia	*R. sp.* 37_24	A0A1Q6T9Z2	A0A1Q6T9Q2	A0A1Q6T9S6	A0A1Q6T9V0	A0A1Q6T9U6
Firmicutes	Clostridia	*R. sp.* AM36-18	A0A374DJM7	A0A374DL85	A0A374DJ85	A0A374DJ30	A0A374DLF3
Firmicutes	Clostridia	*S. termitidis* DSM 10068	A0A1M5YLG7	A0A1M5YK70	A0A1M5YK25	A0A1M5YKA7	A0A1M5YK03
Firmicutes	Clostridia	*S. glycolicus* (DSM 8271/FlGlyR)	F0SVX7	F0SVX6	F0SVX4, F0SVX5	F0SVX2	F0SVX3

Firmicutes	Clostridia	uncultured *Ruminococcus sp.*	A0A1C6B525	A0A1C6B4W3	A0A1C6B550	A0A1C6B4Z7	A0A1C6B537
Firmicutes	Negativicutes	*P. arboris*	A0A1H7BDT3	A0A1H7B4U3	A0A1H7B3V3	A0A1H7BE09	A0A1H7B521
Proteobacteria	Gamma -proteobacteria	*V. aerogenes* CECT 7868	A0A1M6EU64	A0A1M6EU47	A0A1M6EU23	A0A1M6EUJ0	A0A1M6ETU1
Spirochaetes	Spirochaetia	*T. primitia* (ATCC BAA-887/DSM 12427/ZAS-2)	F5YM79	F5YM78	F5YM80	F5YM81	F5YM82
Spirochaetes	Spirochaetia	*T. sp.* CETP13	A0A1L8MYK5	A0A1L8MYG8	A0A1L8MYG9	A0A1L8MYF2	A0A1L8MYG4

Figure S1

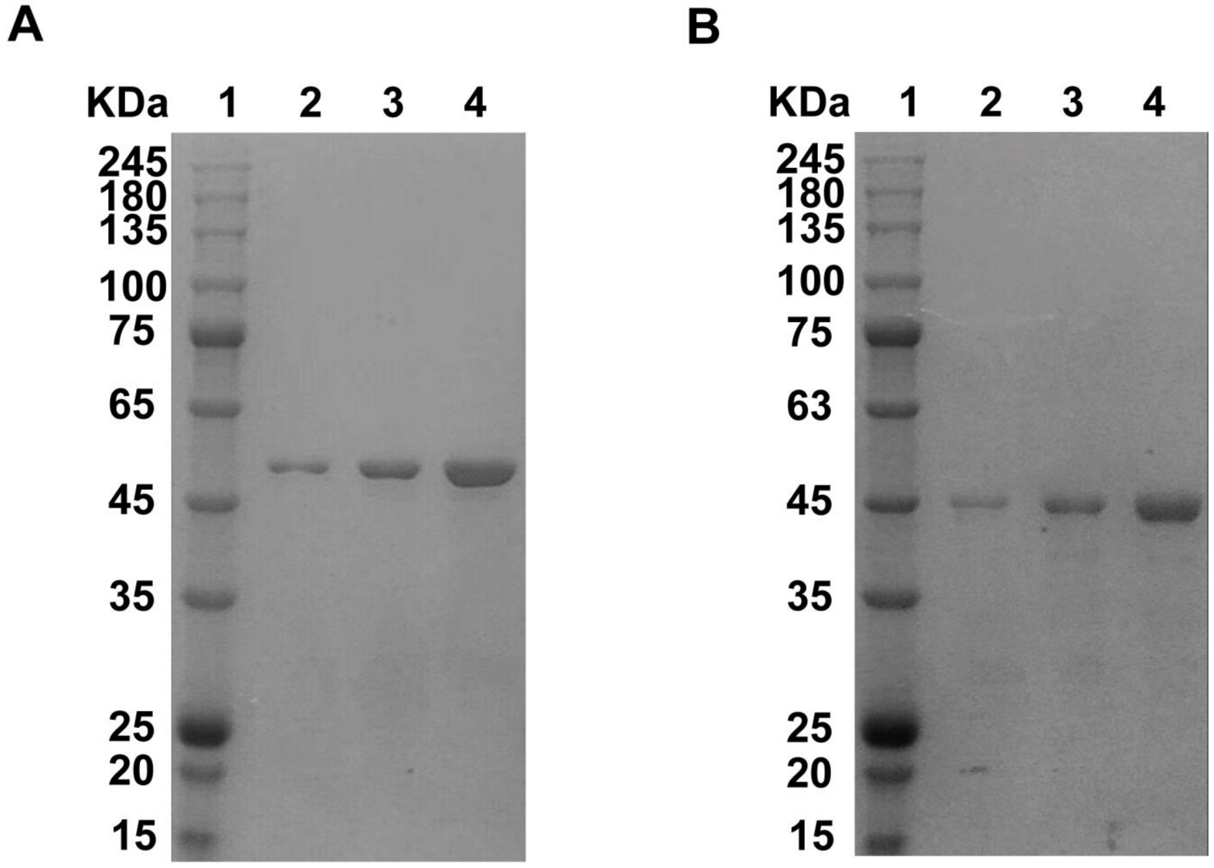


Fig. S1. SDS-PAGE analyses of purified of PydD and PydE. A) PydD. B) PydE. Coomassie-stained 10% SDS gel with: lane 1, molecular weight marker; and lanes 2-4, 1, 2, 4 μg of PydD and PydE.

Figure S2

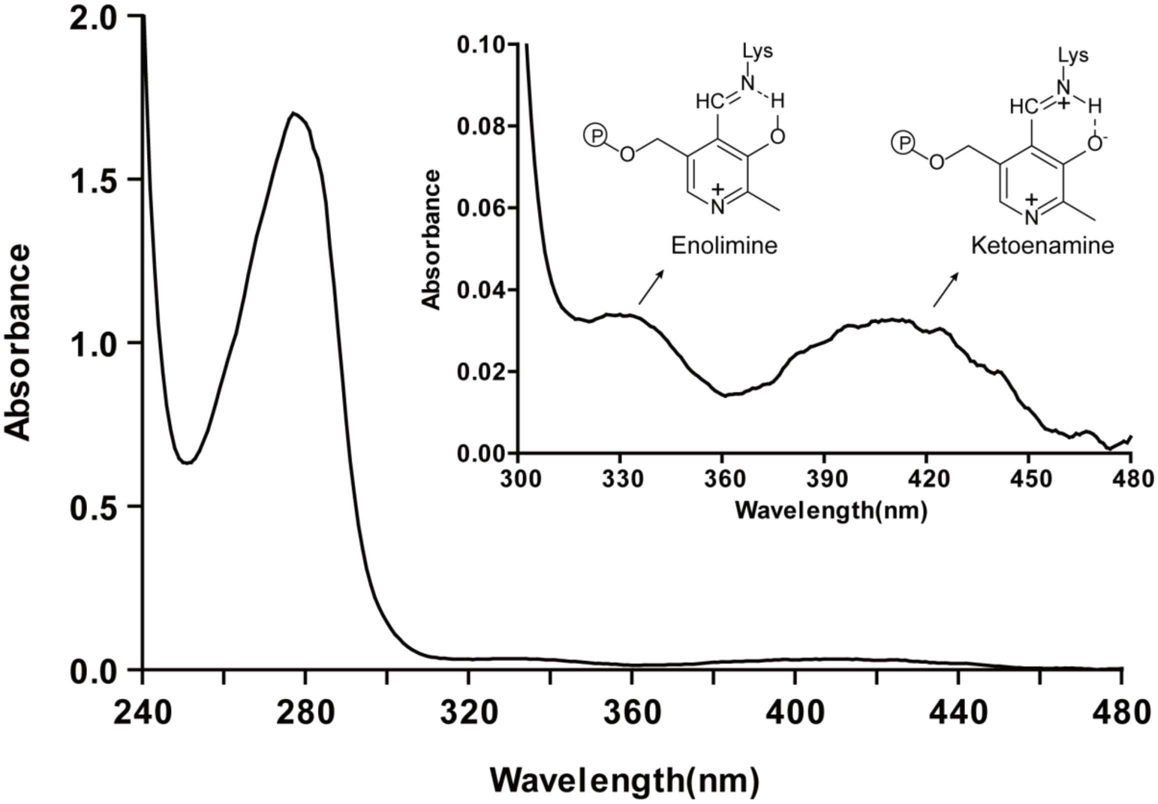


Fig. S2. UV-Vis absorption spectrum of purified PydD. The spectrum was acquired for the eluate from TALON column, in buffer containing 20 mM Tris/HCl, pH 7.5, 0.2 M KCl. Inset: enlargement of the 300-480 nm range, showing the absorbance of the sub-stoichiometric PLP cofactor. The two tautomeric forms of the internal almidine are labeled for the two characteristic peaks.

Figure S3

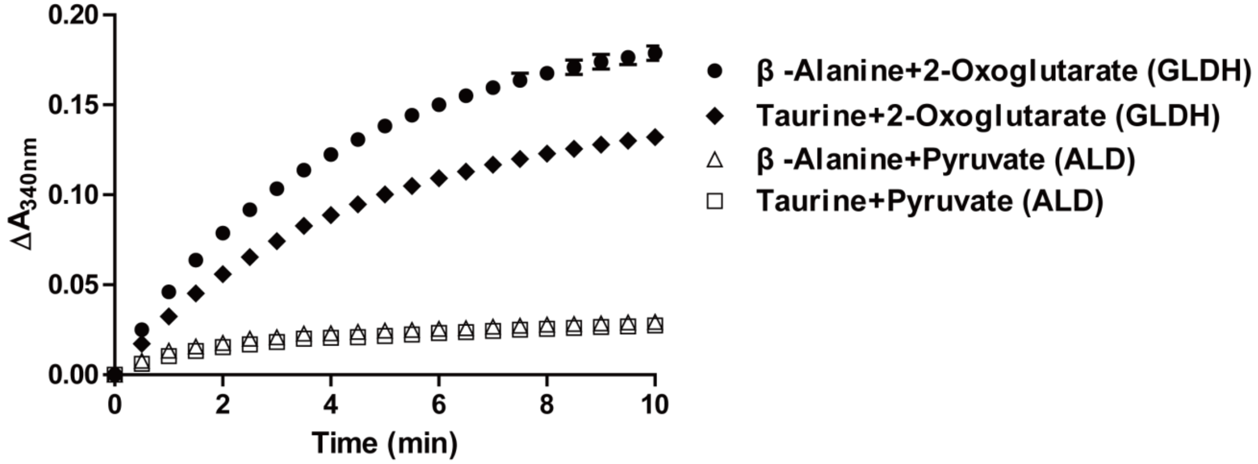


Fig. S3. Substrate analyses of PydD. β-alanine/taurine to 2-oxoglutarate/pyruvate transamination by PydD generates L-glutamate/L-alanine, which is detected by assays monitoring NADH formation accompanying L-glutamate/L-alanine oxidation by GLDH/ALD. The error bars represent the standard error of mean.

Figure S4

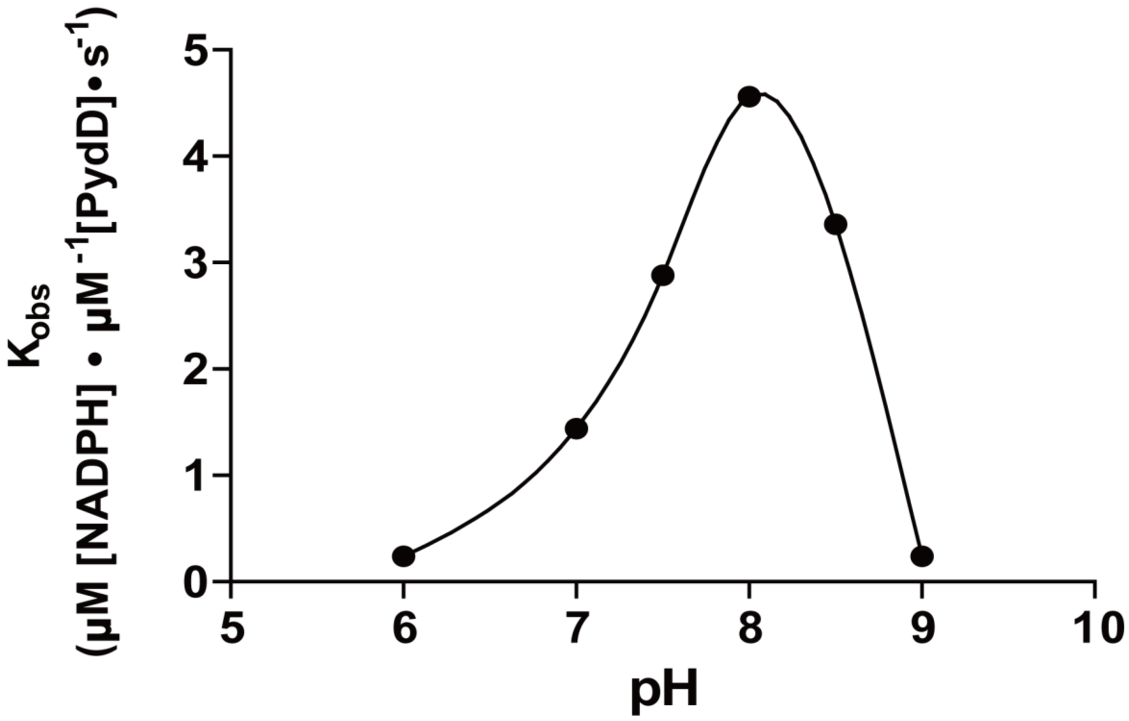


Fig. S4. Effects of pH on PydD activity of malonic semialdehyde reduction. PydE-coupled assay was used. In the presence of excess PydE, the decrease of A_{340} was recorded and used to calculate the activity of PydD under different pH conditions.

Figure S5

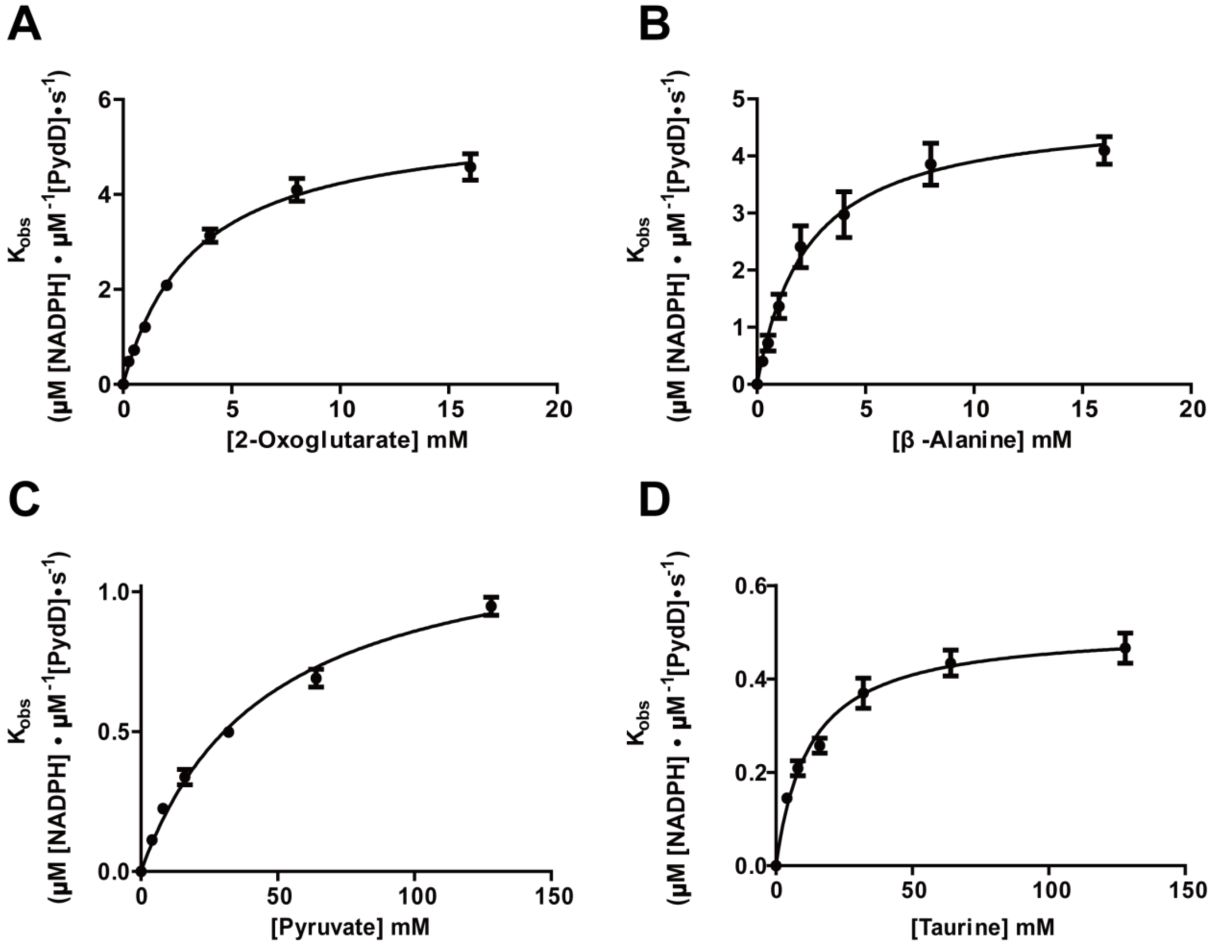
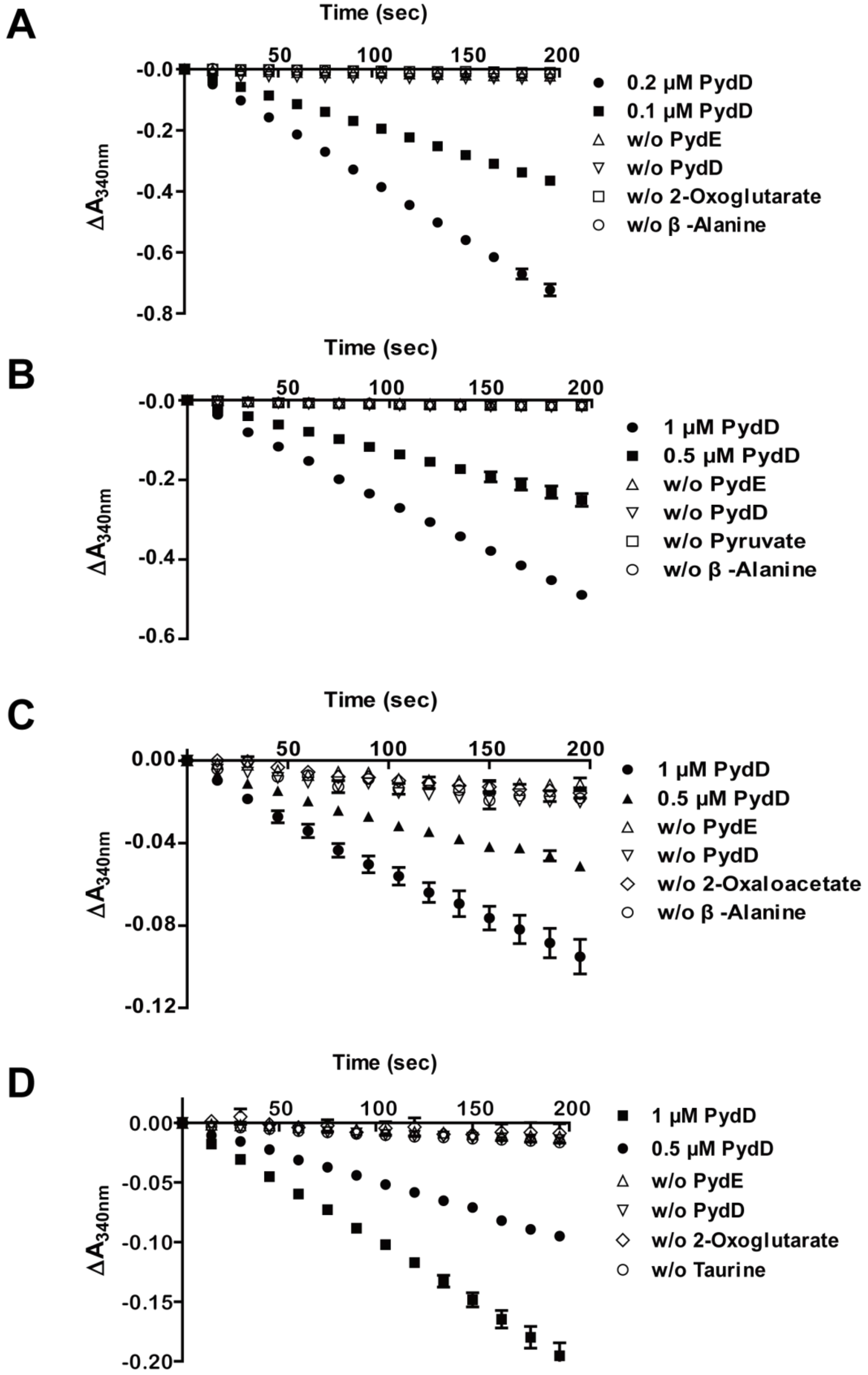


Fig. S5. Michaelis-Menten kinetics of PydD. A) Kinetic assays varying the concentration of the amine acceptor 2-oxoglutarate with a fixed concentration of 25 mM β-alanine. 0.1 μM PydD was used. B) Kinetic assays varying the concentration of β-alanine with a fixed concentration of 25 mM 2-oxoglutarate. 0.1 μM PydD was used. C) Kinetic assays varying the concentration of the amine acceptor pyruvate with a fixed concentration of 25 mM β-alanine. 0.5 μM PydD was used. D) Kinetic assays varying the concentration of taurine with a fixed concentration of 25 mM 2-oxoglutarate. 0.5 μM PydD was used. All assays were performed with the excess of PydE monitoring the decrease of A_{340} representing the consumption of NADPH. The error bars represent the standard error of mean.

Figure S6



A
Time (sec)
50
100
150
200
-0.0
-0.2
-0.4
-0.6
-0.8
ΔA340nm
● 0.2 μM PydD
■ 0.1 μM PydD
△ w/o PydE
▽ w/o PydD
□ w/o 2-Oxoglutarate
○ w/o β -Alanine
B
Time (sec)
50
100
150
200
-0.0
-0.2
-0.4
-0.6
ΔA340nm
● 1 μM PydD
■ 0.5 μM PydD
△ w/o PydE
▽ w/o PydD
□ w/o Pyruvate
○ w/o β -Alanine
C
Time (sec)
50
100
150
200
0.00
-0.04
-0.08
-0.12
ΔA340nm
● 1 μM PydD
▲ 0.5 μM PydD
△ w/o PydE
▽ w/o PydD
◇ w/o 2-Oxaloacetate
○ w/o β -Alanine
D
Time (sec)
50
100
150
200
0.00
-0.05
-0.10
-0.15
-0.20
ΔA340nm
■ 1 μM PydD
● 0.5 μM PydD
△ w/o PydE
▽ w/o PydD
◇ w/o 2-Oxoglutarate
○ w/o Taurine

Fig. S6. Dose-dependent PydD activities on different substrates. A) β-alanine and 2-oxoglutarate. B) β-alanine and pyruvate. C) β-alanine and oxaloacetate. D) taurine and 2-oxoglutarate. All assays were performed with the excess of PydE monitoring the decrease of A_{340} representing the consumption of NADPH. The amounts of PydD used in the assay were as indicated. The error bars represent the standard error of mean.

Figure S7

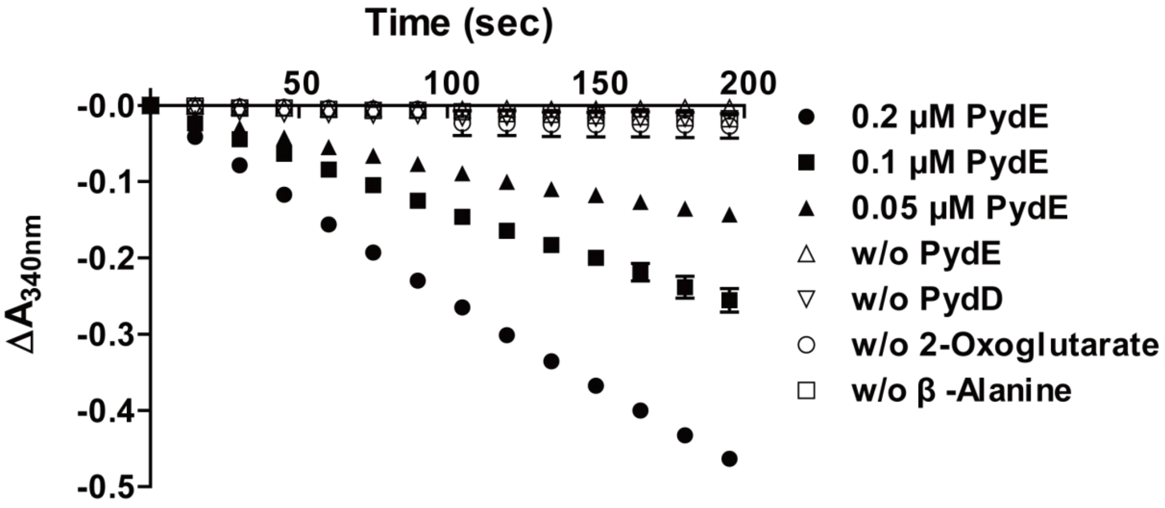


Fig. S7. PydE activity assays. 0.05, 0.1 and 0.2 μM PydE were assayed for activity with 10 μM PydD and 25 mM 2-oxoglutarate and β-alanine. The error bars represent the standard error of mean.

Figure S8

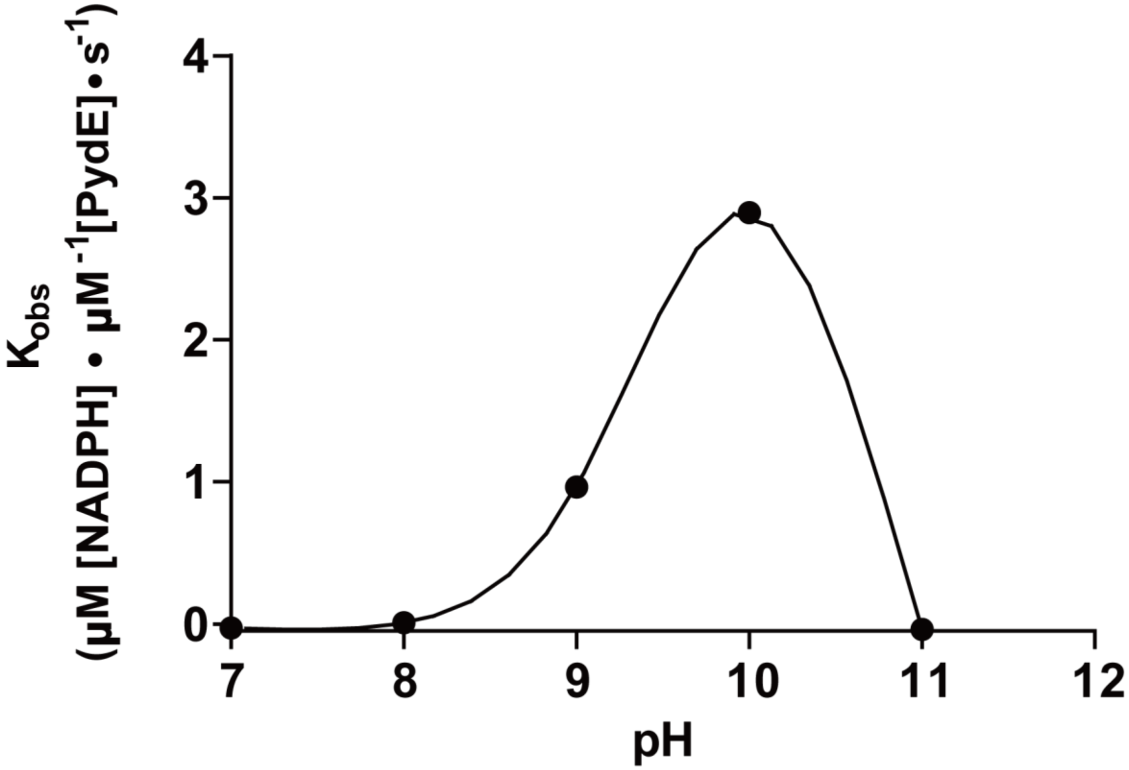


Fig. S8. Effects of pH on PydE activity of 3-HP oxidation. The increase of A_{340} was recorded and used to calculate the activity of PydE under different pH conditions.

Figure S9

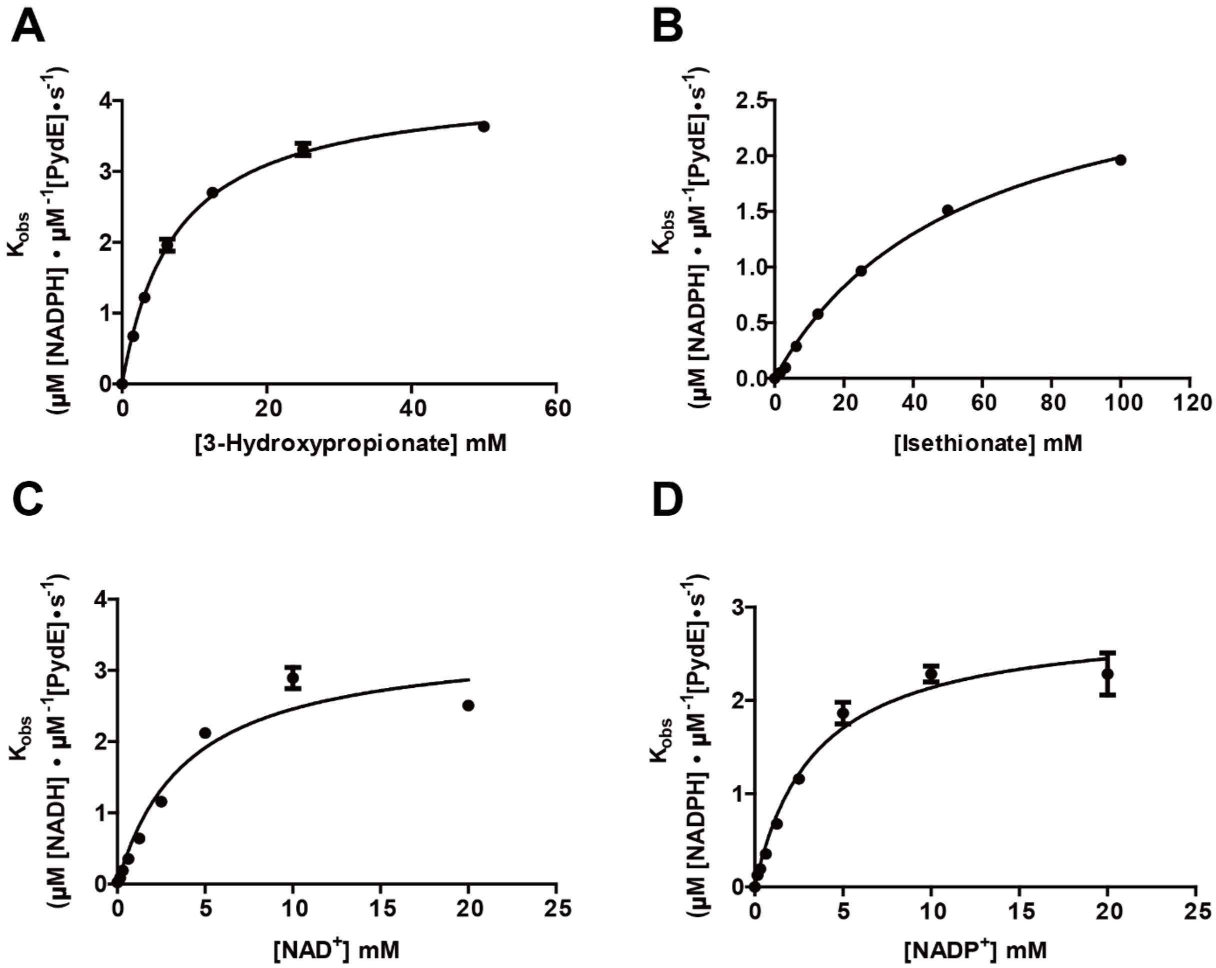


Fig. S9. Michaelis-Menten kinetics of PydE. A) Kinetic assays varying the concentration of 3-HP with a fixed concentration of 15 mM NADP$^+$. B) Kinetic assays varying the concentration of isethionate with a fixed concentration of 15 mM NADP$^+$. C) Kinetic assays varying the concentration of NAD$^+$ with a fixed concentration of 50 mM 3-HP. D) Kinetic assays varying the concentration of NADP$^+$ with a fixed concentration of 50 mM 3-HP. 0.25 µM PydE was used for all assays. The error bars represent the standard error of mean.

Figure S10

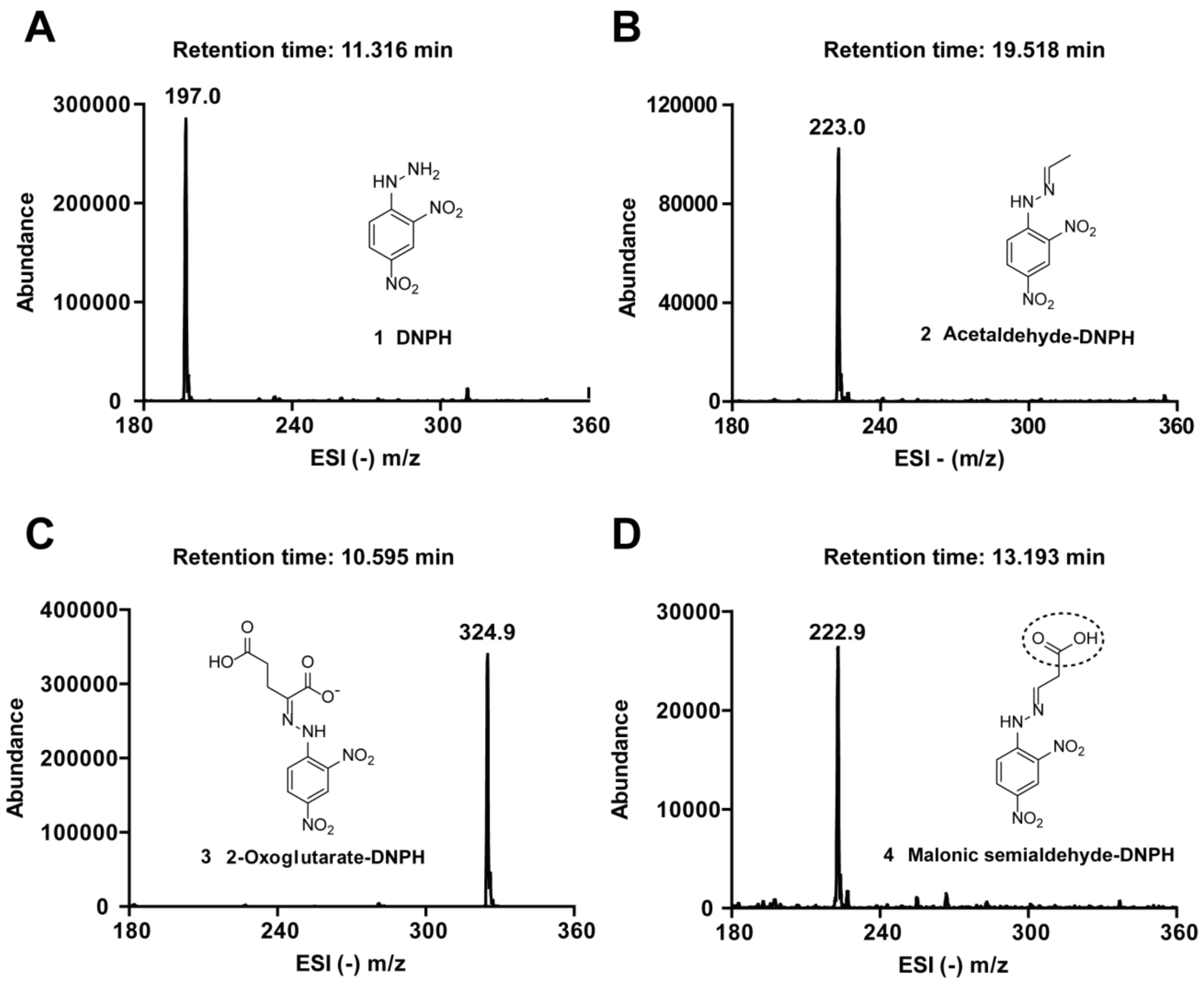


Fig. S10 ESI (-) m/z spectra of the DNPH derivative peaks in Figure 4A. A) Compound **1**. B) Compound **2**. C) Compound **3**. D) Compound **4**.

Figure S11

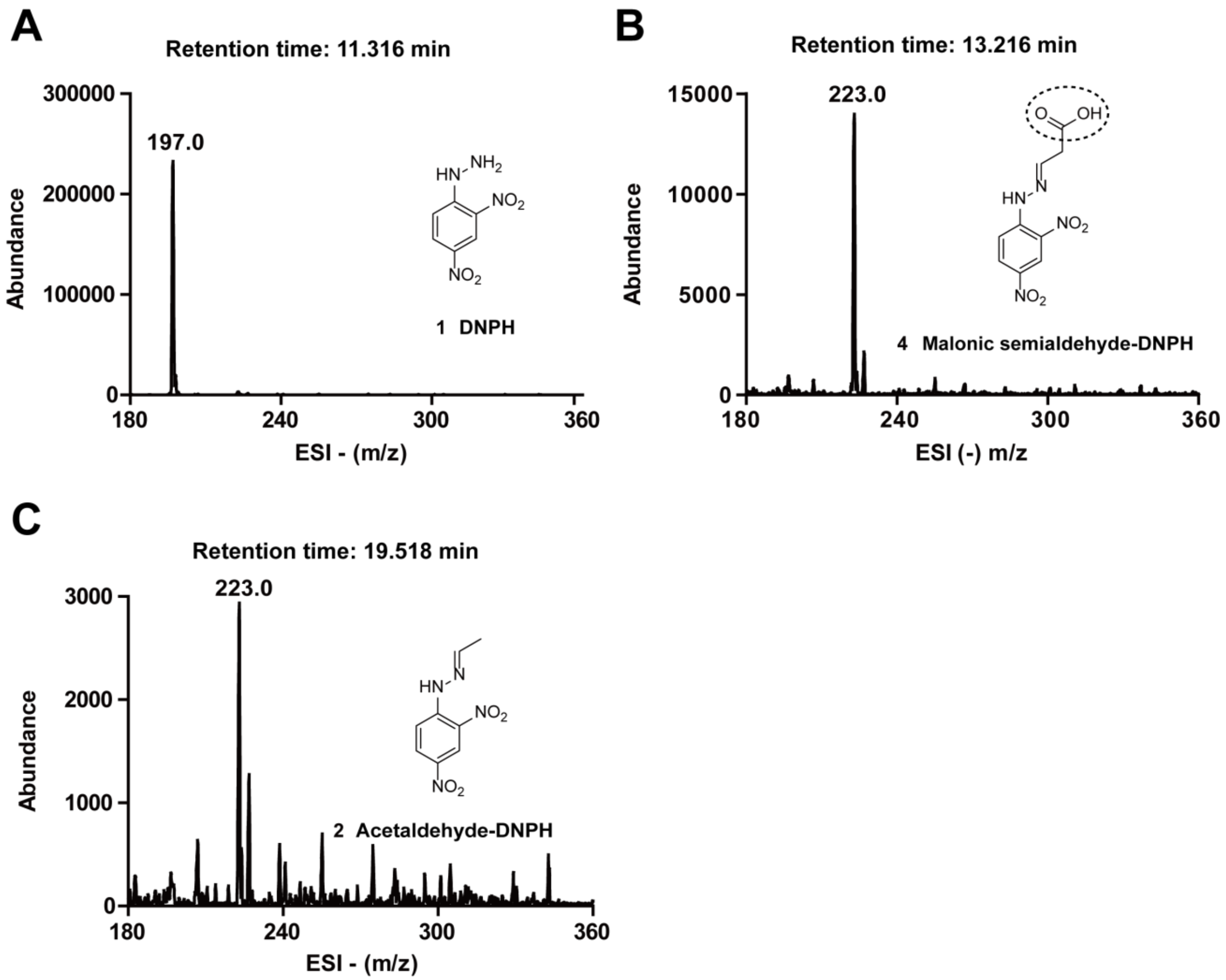


Fig. S11 ESI (-) m/z spectra of the DNPH derivative peaks in Figure 4B. A) Compound **1**. B) Compound **4**. C) Compound **2**.