Supporting Information

Assignment of function to Histidines 260 and 298 by engineering the E1 component of the *Escherichia coli* 2-oxoglutarate dehydrogenase complex; substitutions that lead to acceptance of substrates lacking the 5-carboxyl group.[†]

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[†]Supported at Rutgers by NIH GM050380 (FJ) and at NJIT by NSF MCB0746078 (ETF)

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Materials and Methods

Materials. Thiamin diphosphate (ThDP), dithiothreitol, and isopropyl-β-D-thiogalactopyranoside (IPTG) were from U.S. Biochemical Corp. NAD^+, coenzyme A (CoA), and 2,6-dichlorophenolindophenol (DCPIP) were from Sigma-Aldrich. Restriction enzymes were purchased from Invitrogen.

E. coli strain JW0715 containing the plasmid pCA24N encoding the OGDHc-E1 (E1o) component and AG1 cells containing pCA24N plasmid encoding OGDHc-E2o [both ASKA clone (-)] were obtained from National Bio Resource Project (NIG, Japan).

Site-Directed Saturation Mutagenesis. Site-directed saturation mutagenesis libraries were constructed using a modified QuikChange procedure for H260, H298, and the doubly substituted H260/H298 variants. A typical 50 mL reaction contained 10x PfuUltra buffer, dNTP (0.2 mM) and PfuUltra DNA polymerase (1 unit), pCA24N encoding E1o, and randomization primers:

H298X-F (5'-CGTGAAATACNNSATGGGCTTCTCG-3';

H298X-R (5'-CGAGAAGCCC ATSNNGTATTTCACG-3';

H260X-F (5'CGGGATGGCGNNSCGTGGTCGTC-3';

H260X-R (5'-GACGACCACGSNNCGCCATCCCG -3'.

The PCR reaction consisted of 18 cycles at 95 °C for 30 s, 55 °C for 1 min and 68 °C for 10 min. After the PCR, *Dpn*I was added to the reaction mixture and then PCR product was transformed

into *E. coli* DH5αcells. In order to randomize both positions, the template was the PCR product from H298 randomization and primers were H260X-F and H260X-R.

Adaptation of the E1o specific activity assay for microtiter plates. The E1o specific activity assay was adapted to measure activity in whole cell lysates in a 96-well microtiter plates. The plasmids containing the mutant genes for H260X, H298X or H260X/H298X were transformed into DH5α competent cells and plated onto LB agar plates containing chloramphenicol (100 μg/mL). Single colonies were picked into 96-well microtiter plates containing 200 μL LB medium and chloramphenicol (100 μg/mL). The first column contained wild-type E1o as control. The plates were incubated overnight at 37 °C in the shaker at 250 rpm. The master plates were duplicated using 96-slot pin multi-blot replicator and it was stored at 4 °C. The duplicate plates contained 200 μL LB medium supplemented with chloramphenicol (100 μg/mL), $MgCl_2$ (2 mM), thiamin hydrochloride (1 mM) and IPTG (1 mM). The resulting plates were incubated overnight at 37 °C with shaking at 250 rpm. Next, the plates were centrifuged (1600 *g*) and the medium was discarded. The cell pellets were resuspended with 200 μL NaH_2PO_4 (100 mM, pH 7.0) containing ThDP (1 mM), $MgCl_2$ (2 mM), lysozyme (1 mg/mL), and *DNase*I (0.1 μg/mL). The plates were incubated at 37 °C for 30 min and centrifuged (1600 *g*). The supernatant was assayed for enzymatic activity.

E1o-specific activity was assessed using the external oxidizing agent 2,6-dichlorophenolindophenol (DCPIP); its reduction was measured at 600 nm. The assay mixture contained: 110 μL NaH_2PO_4 (100 mM, pH 7.0), DCPIP (0.1 mM), $MgCl_2$ (2.0 mM), ThDP (1.0 mM), and 2-oxoglutarate (2.0 mM, 2-OG) or 2-oxovalerate (4.0 mM, 2-OV). The reaction was initiated by the addition of 40 μL of supernatant, and active variants were identified from the

disappearance of the blue color on DCPIP reduction. The endpoint absorbance was measured using the microtiter plate reader at 600 nm at 30 °C. A rescreening was performed to eliminate false positives. Each positive clone was retrieved from the master plate and it was streaked on LB agar plates containing chloramphenicol (100 μg/mL). Single colonies from each clone were picked into a column of wells in a microtiter plate, and one column was reserved for the wild-type E1o. The rescreen was similar to the E1o assay described above. The positive clones for 2-OG or 2-OV were sequenced using ABI BigDye Terminator chemistry on an ABI 3130xl genetic analyzer (Molecular Resource Facility, University of Medicine and Dentistry of New Jersey, Newark).

Overexpression and purification of wild type E1o and E1o variants.

An *E. coli* AG1 frozen stock harboring the E1o plasmid was streaked on LB agar plates containing chloramphenicol (100 μg/mL) and incubated at 37 °C overnight. A single colony was used to inoculate 20 mL of LB medium containing chloramphenicol (100 μg/mL). The overnight culture was used to inoculate 1 L of LB medium containing chloramphenicol (100 μg/mL), thiamin hydrochloride (1.0 mM), and $MgCl_2$ (2.0 mM). The culture was induced with IPTG (1.0 mM) and incubated at 30 ^{o}C with shaking overnight. The cells were precipitated at 4400 *g* at 4 °C, and stored at -20 °C.

All subsequent steps were carried out at 4 °C. The cells were resuspended in 20 mM KH_2PO_4 (pH 7.0) containing NaCl (0.1 M), $MgCl_2$ (2.0 mM), ThDP (1.0 mM), benzamidine hydrochloride (1.0 mM), PMSF (1.0 mM), lysozyme (0.6 mg/mL) and incubated on ice for 20 min. The cells were sonicated for 4 min (10 s pulsar "on" and 10 s pulsar "off") using the Sonic Dismembrator Model 550 (Fisher Scientific). The lysate was centrifuged at 30,000 *g* at 4 °C for 30 min. The supernatant was applied to a Ni Sepharose 6 Fast Flow Column (GE Healthcare)

that was equilibrated with KH_2PO_4 (20 mM, pH 7.4), which contained NaCl (0.1 M), $MgCl_2$ (2 mM), ThDP (1 mM), benzamidine hydrochloride (1 mM). The enzyme was eluted with KH_2PO_4 (20 mM, pH 7.4) containing NaCl (0.5 M), imidazole (150 mM), $MgCl_2$ (2.0 mM), and ThDP (1.0 mM). Fractions with enzyme were combined, dialyzed against KH_2PO_4 (20 mM, pH 7.4) containing $MgCl_2$ (2.0 mM), ThDP (1.0 mM) and benzamidine hydrochloride (1.0 mM). Next, the enzyme was concentrated by ultrafiltration with a cutoff of 30 kDa. The purity was confirmed by SDS-PAGE. The E1o variants were overexpressed and purified using the procedure developed for wild-type E1o. Wild type E1o and E1o variants were stored at -20 °C.

Expression and purification of the E2o. A single colony on the plate was used to inoculate 16 mL LB containing chloramphenicol (30 μg/mL). Next, the overnight culture was inoculated into LB (800 mL) containing chloramphenicol (30 μg/mL) and lipoic acid (0.3 mM), and cells were grown at 37 ^{o}C to an OD_{650} = 0.6 - 0.7. The E2o expression was induced by IPTG (0.1 mM) for 5 h at 37 ^{o}C. Cells were collected, washed with KH_2PO_4 (20 mM, pH 7.0) containing NaCl (0.15 M) and stored at -20 ^{o}C.

The E2o was purified, as was E2p (*1*) with some modifications. The precipitation of E2o by ammonium sulfate was replaced by ultracentrifugation at 117,426 g for 4 h. E2o was purified using Sephacryl S-300 HR gel-filtration column equilibrated with 20 mM KH_2PO_4 (pH 7.2) containing 0.50 mM EDTA, 0.20 M NaCl, 1 mM DTT and 1 mM benzamidine HCl. Fractions containing E2o were collected and protein was precipitated by ultracentrifugation as described above. The pellet was dissolved in 20 mM KH_2PO_4 (pH 7.2) containing 0.50 mM EDTA, 1 mM DTT, 1 mM benzamidine HCl and 0.50 mM NaCl (the concentration of NaCl was increased from 0.2 M to 0.5M to keep protein soluble). E2o was stored at -80 ^{o}C.

Expression and Purification of E. coli E3 was carried out as in ref (*2*).

Activity and related measurements. The E1-specific activity of wild type E1o and its variants was measured by monitoring the reduction of DCPIP at 600 nm using a Varian DMS 300 or DMS 500 spectrophotometer. The assay medium contained in KH_2PO_4 (20 mM, pH 7.0): $MgCl_2$ (2 mM), ThDP (0.2 mM), DCPIP (0.1 mM) and 2-OG (2 mM) or pyruvate (25 mM) or 2-OV (45 mM) at 30 °C. The reaction was initiated by adding the enzyme. One unit of activity is defined as the amount of DCPIP reduced (μmol/min/mg of E1o).

The overall activity of the OGDHc was determined from the substrate-dependent reduction of NAD^+ at 340 nm after reconstitution for 20 min at 25 ^oC of E1o with E2o and E3 at a mass ratio of 1:1:1 (mg/mg/mg) in 20 mM KH_2PO_4 (pH 7.2) containing 0.15 M NaCl. The assay medium contained: 0.1 M Tris-HCl (pH 8.0), $MgCl_2$ (1.0 mM), ThDP (0.2 mM), dithiothreitol (2.6 mM), NAD^+ (2.5 mM), and substrate [2-OG (2 mM) or pyruvate (25 mM) or 2-OV (45 mM)]. The reaction was initiated by the addition of CoA (0.13 mM). The initial steady state velocities were determined from the progress curves recorded at 340 nm and 30 ^oC. One unit of activity is defined as the amount of NADH produced (μmol/min/mg of E1o). The reaction conditions for the hybrid E1o-E2p-E3 and E1p-E2o-E3 complexes were the same. It should be noted that the E3 component is common to PDHc and OGDHc from *E. coli* (*3,4*).

Circular Dichroism (CD). CD experiments were carried out on a Chirascan CD spectrometer (Applied Photophysics, Leatherhead, UK) at 30 ^oC by using the pyruvate and 2-OG analogs sodium acetylphophinate (AcP^-) (*5*), methyl acetylphosphonate (MAP^-) (*6*), propionyl-phosphinate (PP^-), succinylphosponate (SP^{2-}) and succinylphosphonate methyl ester ($SPME^-$) (see Supporting Information for synthesis). E1o and its variants (concentration of active centers = 20 - 24 μM) in 20 mM KH_2PO_4 (pH 7.0) containing NaCl (0.15 M), $MgCl_2$ (2.0 mM) and

ThDP (0.2 mM) were titrated with substrate analogs (0.5 – 2000 μM). The difference CD spectra were obtained by subtracting the CD spectrum of E1o or its variants in the absence of substrate analogs. The values of K_d were determined as described earlier (*7*).

Sample Preparation for Fourier Transform Mass Spectrometric Analysis (FTMS). Electro-spray ionization FTMS was performed with an Apex-ultra 70 hybrid FTMS instrument from Bruker Daltonics (Billerica, MA). The E1p-E2o-E3 or E1o-E2p-E3 hybrid complexes were reconstituted by the same method as above. Sequentially, the complexes were incubated for 10 min in the reaction medium containing: Tris-HCl (0.1 M, pH 8.0), $MgCl_2$ (1.0 mM), ThDP (0.2 mM), dithiothreitol (2.6 mM), NAD^+ (2.5 mM), CoA (0.13 mM) and 2-OG (2 mM) or pyruvate (2 mM) at 25 ^oC. To quench the reaction and precipitate protein, an acidic quench solution consisting of 12.5% trichloroacetic acid/1 M HCl was added. After the acid quench, protein was removed by centrifugation at 15,700 *g* for 15 min. For control, acetyl-CoA, succinyl-CoA and CoA stock solutions were prepared in deionized water and diluted to 0.13 mM in acidic quench solution. Then, 50 μL of each sample was mixed with 50 μL of solvent containing 0.1 % (v/v) formic acid and 50 % MeOH (v/v) in deionized water. These samples were then injected into FTMS at 2 μL/min; 20 scans were acquired with a netflow rate of 1.0.

Sample preparation for detection of acetylated and succinylated lipoyl domain (LD) is presented in Supporting Information.

Synthesis of phosphonate analogs of 2-oxoglutarate *(8).*

Ethyl 4-(dimethylphosphono)-4-oxobutanoate. To trimethyl phosphite (4.75 mL, 40.3 mmol) under argon was added ethyl succinyl chloride (6.34 mL, 44.3 mmol) dropwise. The resultant solution was stirred at room temperature for 7 h. The biproducts were removed by distillation (below 85 ^oC, 0.050 mm Hg), and the ethyl 4-(dimethylphosphono)-4-oxobutanoate was

collected by distillation (98-100 ^{o}C, 0.050 mm Hg, 8.2 g, 86.3%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 4.12 (q, *J* = 7, 7.5 Hz, 2 H), 3.87 (s, *J* = 11 Hz, 6 H), 3.13 (t, *J* = 2.5 Hz, 2 H), 2.62 (t, *J* = 6, 6.5 Hz, 2 H), 1.23 (t, *J*= 7, 7.5 Hz, 3 H).

Ethyl 4-(sodium, methylphosphono)-4-oxobutanoate. To a solution of ethyl 4-(dimethylphosphono)-4-oxobutanoate (2.0 g, 8.40 mmol) in acetone (25 mL) under argon was added dropwise NaI (1.51 g, 10.1 mmol) in 5 mL acetone over the course of 10 min. The resultant solution was heated to reflux and stirred for 24 h. The solvent was removed in vacuo to afford the ethyl 4-(sodium, methylphosphono)-4-oxobutanoate as a pale yellow solid, which was > 99% pure as determined by ^{1}H NMR analysis. This material was used in the next reaction step without further purification: ^{1}H NMR (D_2O) δ 4.01 (q, *J* = 7, 7.5 Hz, 2 H), δ 3.46 (m, *J* = 9 Hz, 3 H), δ 3.01 (t, *J* = 6 Hz, 2 H), δ 2.50 (t, *J* = 6 Hz, 2 H), δ 1.09 (t, *J* = 6.5, 1 Hz, 3 H).

Sodium 4-(sodium, methylphosphono)-4-oxobutanoate. To ethyl 4-(sodium, methylphosphono)-4-oxobutanoate (2.0 g, 8.12 mmol) was added 1 N NaOH (8.3 mL, 8.12 mmol) and the resultant solution was stirred at room temperature for overnight. The water was removed in vacuo to afford the sodium 4-(sodium, methylphosphono)-4-oxobutanoate as a pale yellow solid, which was 92.30% pure as determined by ^{1}H NMR analysis: (D_2O) δ 3.45 (s, *J* = 11 Hz, 3 H), 2.90 (t, *J*= 6.5 Hz, 2 H), 2.24 (t, *J* =7 Hz, 2 H) ppm; ^{13}C NMR (D_2O) δ 220.3, 181.1, 52.8, 39.9, 29.8 ppm.

Synthesis of sodium propionylphosphinate

Sodium (1,1-diethoxypropyl)phosphinate (9). Hypophosphorous acid (50% w/v in H_2O) (6.6 mL, 0.05 mol) was evaporated at 0.01 mm Hg to remove water using a 50 mL Schlenk flask kept in an ice bath connected to a trap (kept at – 70 ^{o}C). Triethyl orthopropionate (20.5 g, 0.116 mol)

was added in a drop-wise manner to the anhydrous acid under N_2 on an ice bath. Under vigorous stirring, dry HCl was passed through the solution until it became cloudy. The resulting mixture was allowed to warm to room temperature and was stirred overnight under N_2. To remove the volatiles the reaction mixture was evaporated under reduced pressure. After complete removal of all the volatile material, a solution of NaOH (3 g, 0.15 mol) in 30 mL degassed water was added in a drop-wise manner to the reaction mixture kept on an ice bath. The reaction mixture was then refluxed under N_2 for 2 h. Next, the reaction mixture was allowed to cool down and the solution pH was adjusted to 8.0 with concentrated HCl on an ice bath. The solution was evaporated to dryness and the residue was triturated with 60 mL of boiling absolute ethanol. After filtration, ethanol was evaporated and the residue was recrystallized in absolute ethanol. Yield (1.35 g, 14.1 %). ^{1}H NMR (500 MHz, D_2O-DSS): δ 6.934 (d, J = 515.5 Hz, 1 H), 3.689 (q, J = 6.5 Hz, 4 H), 1.831 (q, J = 6.5 Hz, 2H), 1.173 (t, J = 7 Hz, 6 H), 0.957 (t, J = 7 Hz, 3 H).

Sodium propionylphosphinate (PP^-) Sodium (1,1-diethoxyethyl)phosphinate (1.0 g, 5.2 mmol) was dissolved in a mixture of 9.5 mL of glacial acetic acid and 0.5 mL of water. The solution was stirred at room temperature for 24 h. After removing the solvent in vacuo the residue was triturated with acetone and the resulting white solid was recrystallized from methanol/acetone. Yield (245 mg, 36.2 %). ^{1}H NMR (500 MHz, D_2O-DSS): δ 6.789 (d, J = 544 Hz, 1 H), 2.809 (m, J = 6 Hz, 3 Hz, 2 H), 1.035 (t, J = 7 Hz, 3 H).

Reductive acetylation of the LD-E2p detected by FTMS.

The rate constant of the reductive acetylation of the LD-E2p by E1o was determined under single-turnover conditions. The 15 μL of the reaction mixture containing E1o (28 μM) and LD-E2p (28 μM) in 50 mM ammonium bicarbonate (pH 7.0) supplemented with ThDP (0.40 mM)

and $MgCl_2$ (2.0 mM) was mixed with 15 μL of pyruvate (4 mM stock solution) in a total volume of 30 μL. The reaction was quenched at different times by addition of 83 μL of 50% methanol and 2% formic acid. The resulting samples containing LD-E2p at 14 μM were diluted to 1 μM concentration with 50% methanol and 2% formic acid. The ratio of acetylated LD-E2p to a total LD-E2p (acetylated plus un-acetylated) was plotted against time and yielding a rate constant (Details will be published elsewhere).

Reductive succinylation of the LD-E2p detected by FTMS.

For reductive succinylation of LD-E2p a reaction mixture consisting of 20 μM LD-E2p in 20 mM $(NH_4)_2CO_3$ (pH 7.0), containing 1 mM $MgCl_2$, 0.1 mM ThDP, 1 mM α-ketoglutaric acid (sodium salt), and 1 μM *wt* E1o was incubated for 15 min at 25°C. LD-E2p samples were diluted to 1 μM final concentration with 49:49:2 (v/v/v) mixture of methanol, water and formic acid.

References

1. Song, J., Park, Y-H, Nemeria, N.S., Kale, S., Kakalis, L., Jordan, F. (2010) *J. Biol.Chem.28,* 4680-4694.

2. Wei, W., Li, H., Nemeria, N., and Jordan, F. (2003) *Protein Expression and Purification 28*, 140-150.

3. Perham, R. N., Jones, D. D., Chauhan, H. J., and Howard, M. J. (2002) *Biochem. Soc. Trans. 30*, 47-51.

4. Jones, D. D., and Perham, R. N. (2008) *Biochem. J. 409*, 357-366.

5. Nemeria, N.S., Korotchkina, L. G., Chakraborty, S., Patel, M. S., and Jordan, F. (2006) *Bioorg.*

Chem. 34, 362-379.

6. Kluger, R., and Wasserstein, P. (1973) *J. Am. Chem. Soc. 95*, 1071-1074.

7. Nemeria, N., Yan, Y., Zhang, Z., Brown, A. M., Arjunan, P., Furey, W., Guest, J. R., and Jordan, F. (2001) *J. Biol. Chem. 276*, 45969-45978.

8. Bunik, V. I., Denton, T. T., Xu, H., Thompson, C. M., Cooper, A. J. L., and Gibson, G. E. (2005) *Biochemistry 44*, 10552-10561.

9. Ballie, A. C., Wright, B. J., and Wright, K. (1982) Compounds and compositions, (Limited, F., Ed.).

Table S1. Steady-state kinetic parameters for the overall OGDHc activity with 2-OG[a].

Substitution	**NAD^+ activity** ($\mu mol.min^{-1}.mg^{-1}$)	**k_{cat}** (s^{-1})	**K_m** (mM)	**k_{cat}/K_m** ($s^{-1}\ mM^{-1}$)
none	16.6 ± 0.44	57.5 ± 1.57	0.09 ± 3.61	639
H298L	3.80 ± 0.21	13.2 ± 0.51	0.27 ± 0.34	48.9
H298T	0.26 ± 0.01	0.90 ± 0.02	0.23 ± 0.04	3.91
H298D	0.2 ± 0.003	0.79 ± 0.01	0.30 ± 0.03	2.63
H298V	0.28 ± 0.03	0.98 ± 0.09	0.15 ± 0.01	6.53
H260E/H298N	0.28 ± 0.01	0.97 ± 0.03	0.22 ± 0.02	4.41
H260E	0.40 ± 0.01	1.39 ± 0.02	0.72 ± 0.01	1.93

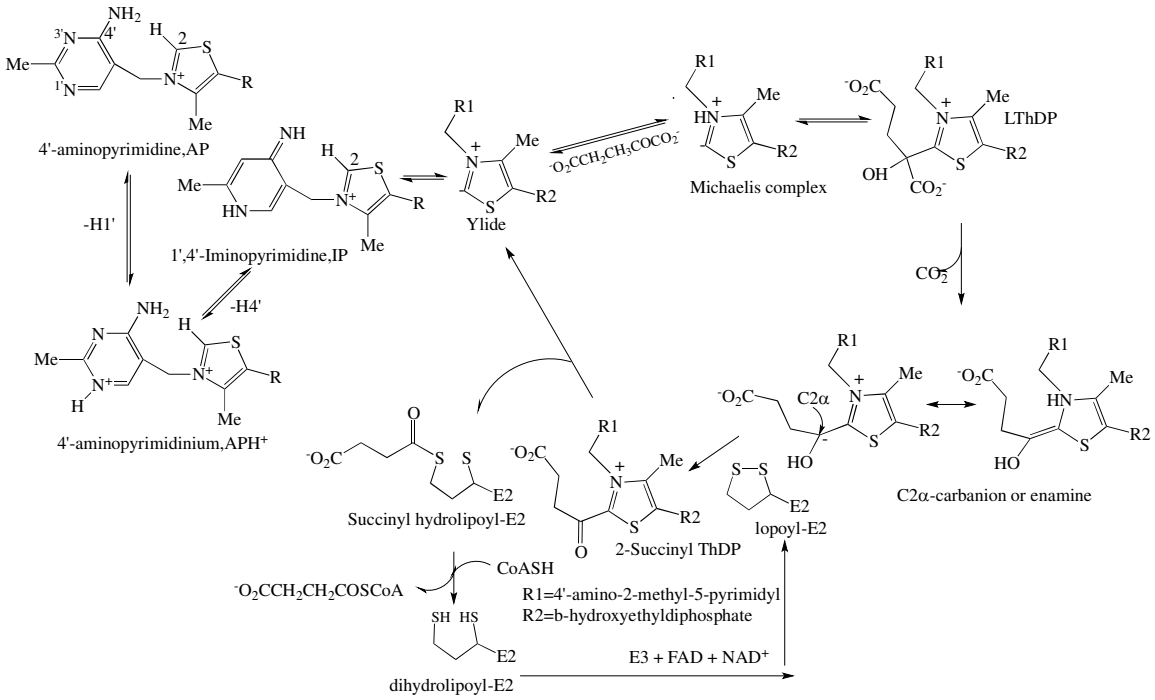
[a]OGDHc variant complexes were assembled from the indicated E1o variants with E2o+E3.

Table S2. Circular dichroism determination of the formation of pre-decarboxylation intermediate between E1o variants and substrate analogs.

E1o substitution	**Substrate analogs**	**CD maximum (nm)**	**K_d (μM)**
wt	AcP^-	297	320 ± 52.2
wt	MAP	No band detected	-
wt	PP^-	300	39.0 ± 2.08
wt	SP^{2-}	300	28.4 ± 1.12
wt	$SPME^-$	297	10.1± 0.42
H298L	$SPME^-$	297	Not saturated
H298L	PP^-	300	22.3 ± 0.98
H298T	$SPME^-$	297	6.07 ± 0.53
H298T	PP^-	297	7.00 ± 0.23
H298D	$SPME^-$	298	659 ± 11.5
H298D	PP^-	300	9.61 ± 0.20
H298V	$SPME^-$	297	144 ± 11.6
H298V	PP^-	297	5.17 ± 1.04
H260E/H298N	$SPME^-$	No band detected	-
H260E/H298N	PP^-	305	7.69 ± 2.50
H260E	$SPME^-$	No band detected	-
H260E	PP^-	No band detected	-

AcP^-, acetylphophinate; MAP, methyl acetylphosphonate; PP^-, propionylphosphinate; SP^{2-}, succinylphosponate; $SPME^-$, succinylphosphonate methyl ester.

Scheme S1.



Scheme 1. Mechanism of *E.coli* 2-oxoglutarate dehydrogenase complex with role of ThDP.

Figure S1. Near-UV CD spectra of E1o in the presence of pyruvate. The E1o (13.2 μM active center concentration) in 20 mM KH_2PO_4 (pH 7.0) containing 2.0 mM $MgCl_2$ and 0.20 mM ThDP (curve 1). CD spectra of E1o after addition of 4 mM pyruvate at different times of incubation (curves 2-10). Inset: The dependence of the CD at 300 nm on time of E1o incubation with 4 mM pyruvate.

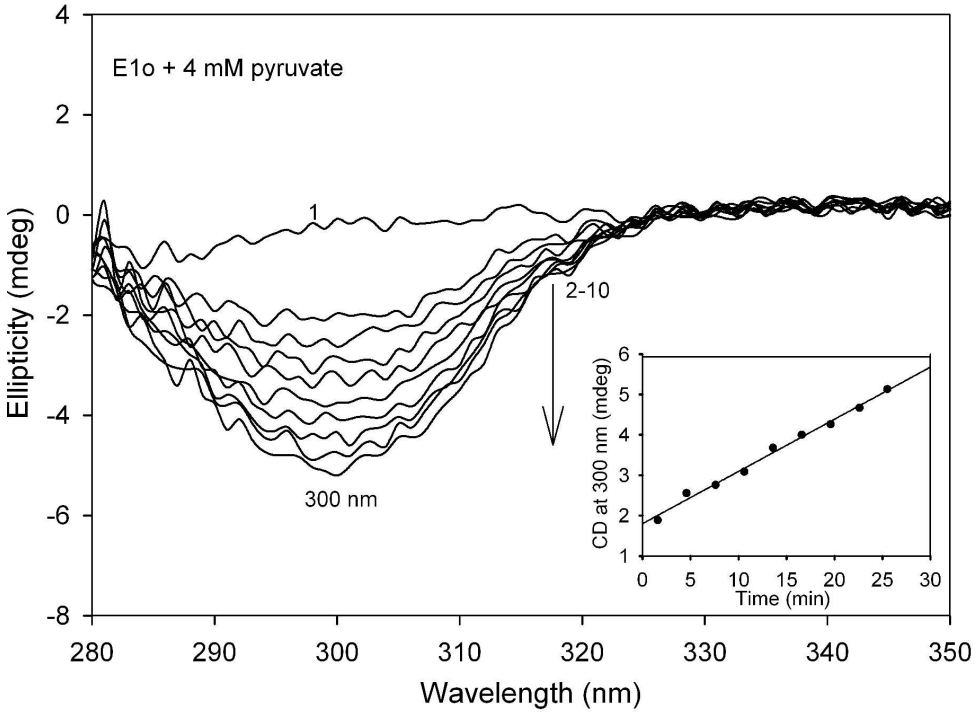


Figure S2. Mass spectrum of succinyl-CoA standard (top) and as produced by E1o-E2o-E3 with 2-oxoglutarate (bottom). For details of experiment see Materials and Methods of the Supporting Information.

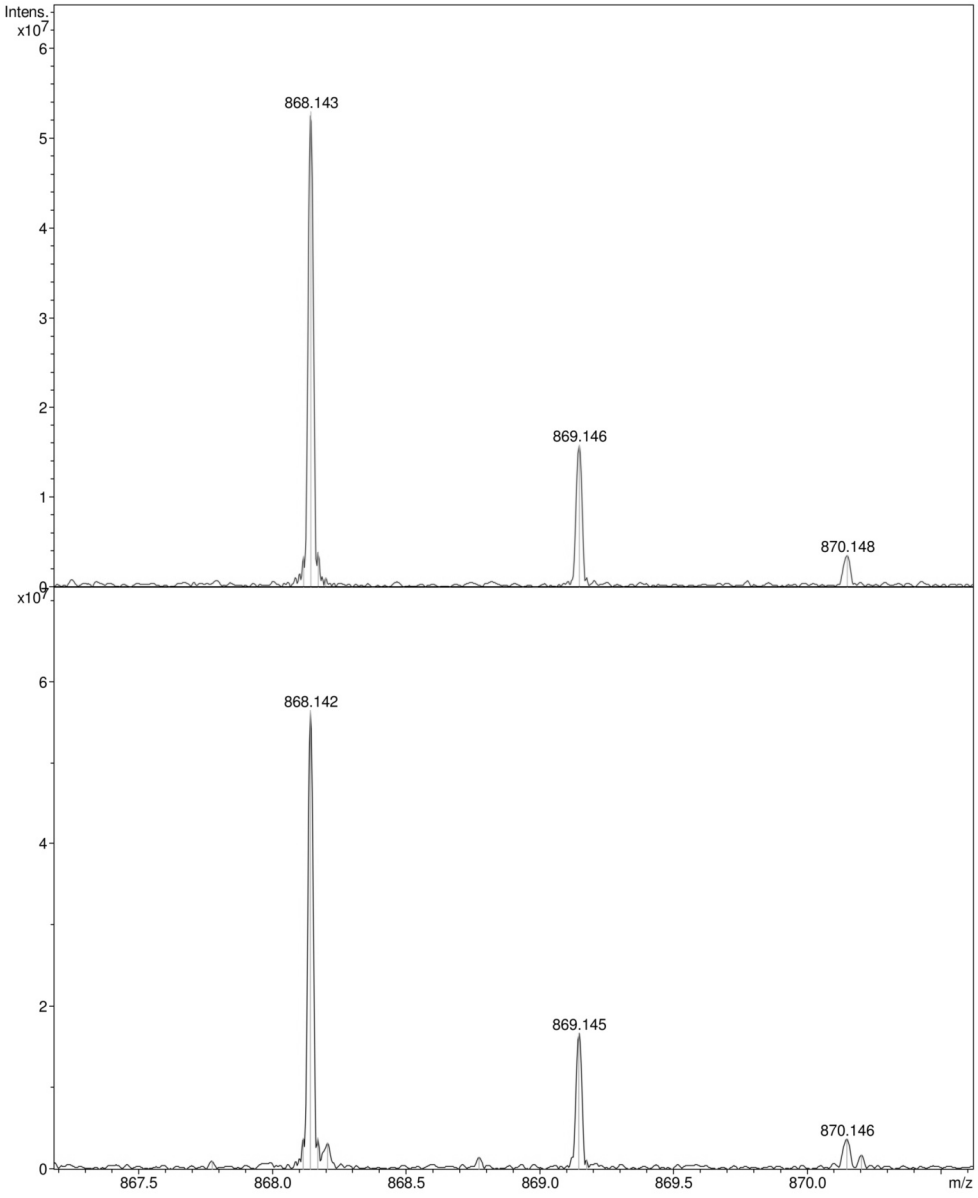


Figure S3. Mass spectrum of succinyl-CoA standard (top) and as produced by E1o-E2p-E3 with 2-oxoglutarate (bottom). For details see Materials and Methods of the Supporting Information.

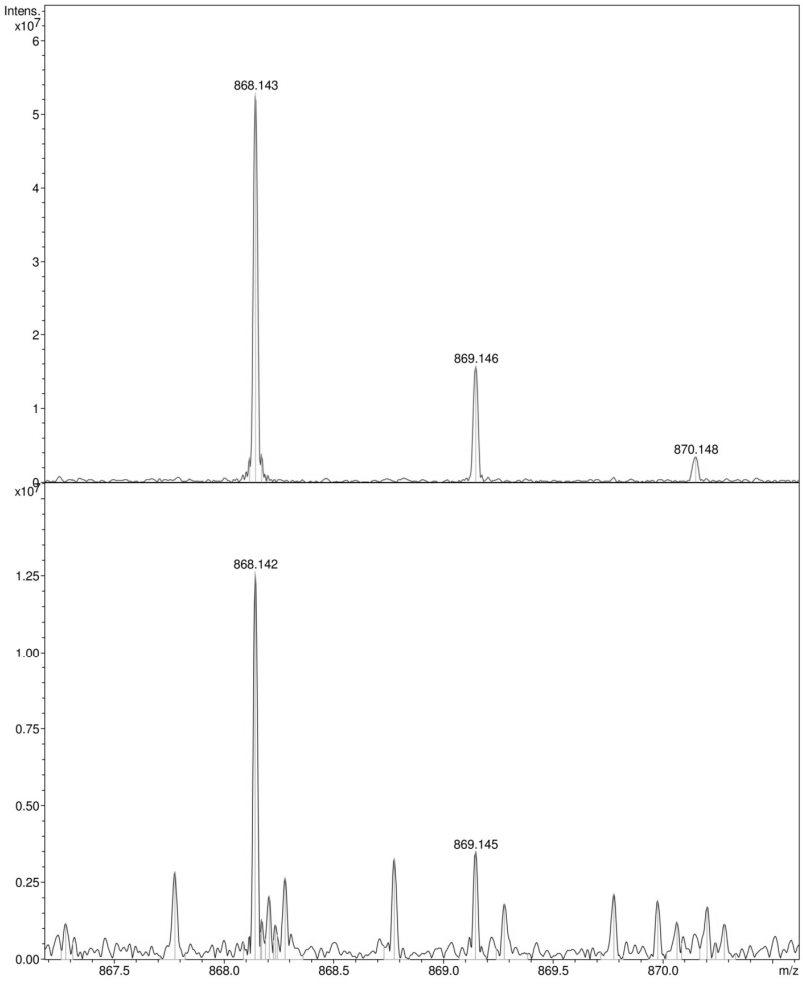


Figure S4. Phosphonate and phosphinate analogs used in experiments.

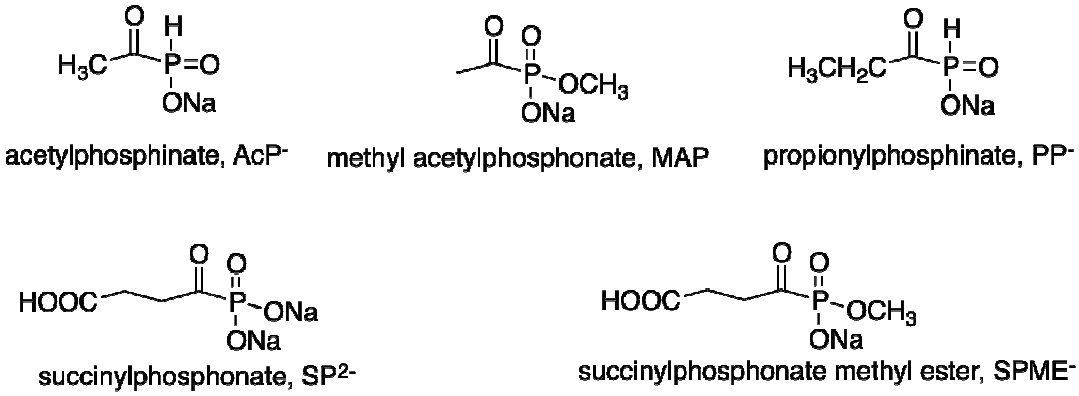


Figure S5A. Circular dichroism titration of E1o by acetylphosphinate. E1o (1.62 mg/mL, concentration of active centers = 15.5 μM) was titrated by AcP^- (5-800 μM) in 20 mM KH_2PO_4 (pH 7.0) containing 2 mM $MgCl_2$ and 0.2 mM ThDP at 30 °C. Inset: Dependence of the ellipticity at 297 nm on concentration of AcP^-.

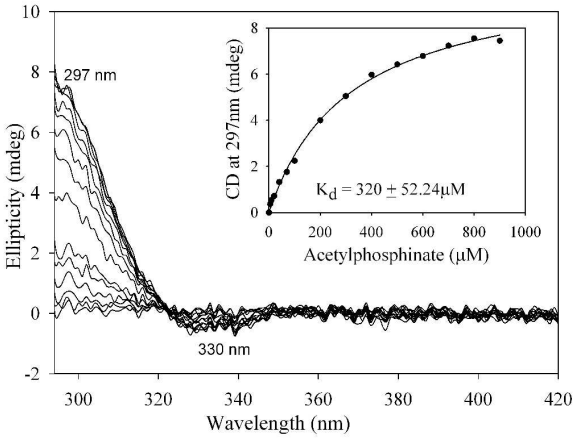


Figure S5B. Circular dichroism titration of E1o with succinylphosphonate methyl ester. E1o (2.5 mg/ mL, concentration of active centers = 23.6 μM) in 20 mM KH_2PO_4 (pH 7.0) was titrated by $SPME^-$ (1-200 μM). in 20 mM KH_2PO_4 (pH 7.0) containing 2 mM $MgCl_2$ and 0.20 mM ThDP. Inset: Dependence of ellipticity at 300 nm on the concentration of $SPME^-$.

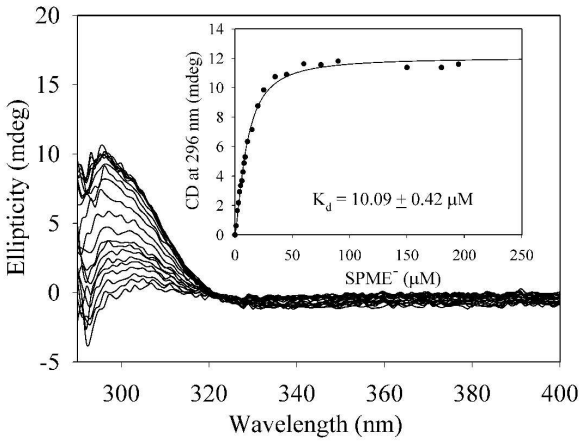


Figure S5C. Circular dichroism titration of E1o with propionylphosphinate. E1o (2.39 mg/mL, concentration of active centers = 22.6 μM) in 20 mM KH_2PO_4 (pH 7.0) containing 2 mM $MgCl_2$, 0.20 mM ThDP and 1.5 mM NaCL was titrated by PP- (30-140 μM). Inset: Dependence of the ellipticity at 297 nm on concentration of PP^-.

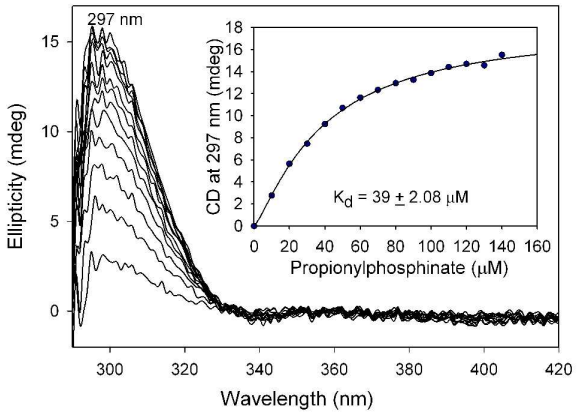


Figure S6. Dependence of the ellipticity at 303 nm on concentration of succinylphosphonate methyl ester for H298L E1o variant.

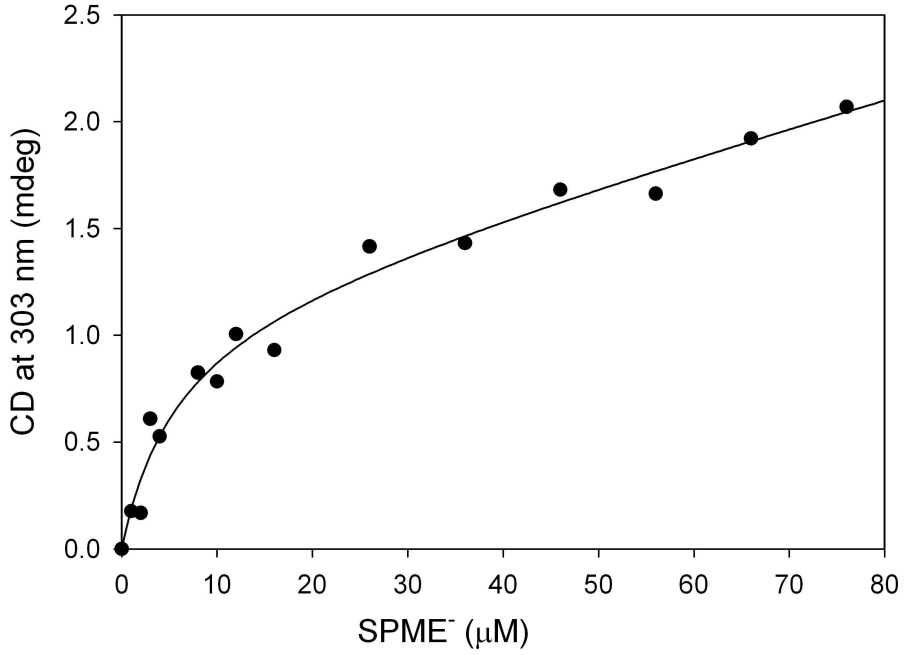


Figure S7. Dependence of the ellipticity at 297 nm on concentration of succinylphosphonate methyl ester for H298T E1o variant.

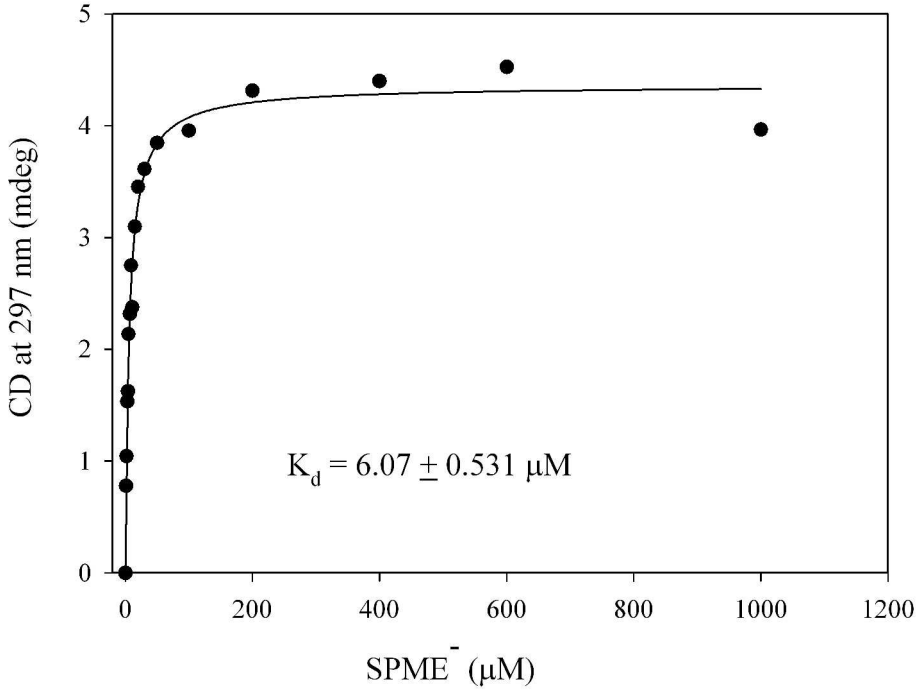


Figure S8. CD titration of H298D E1o with succinylphosphonate methyl ester.

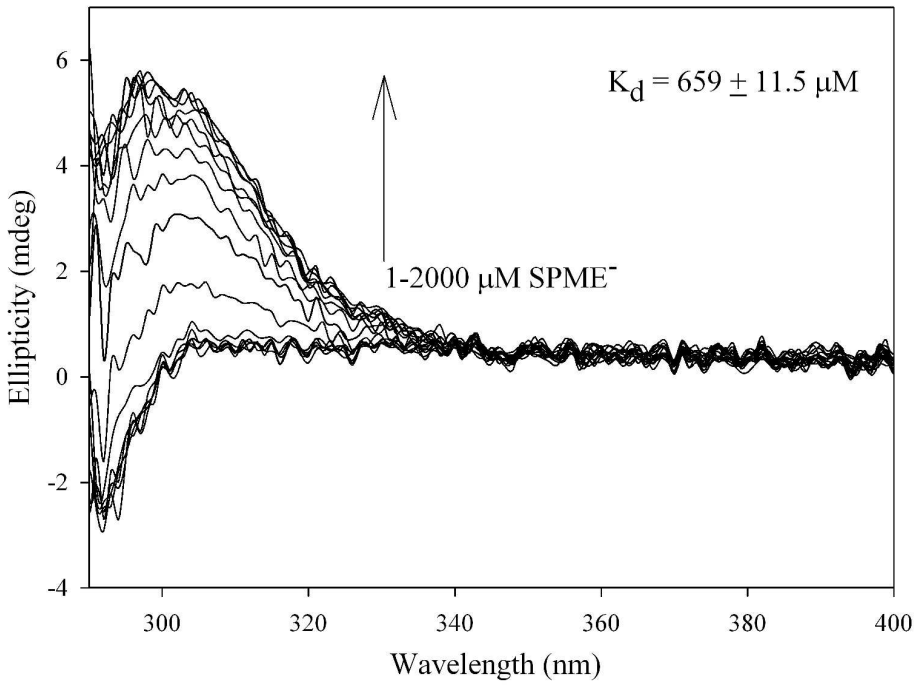


Figure S9. Circular dichroism titration of H298V E1o with succinylphosphonate methyl ester (A). The dependence of the ellipticity at 297 nm on concentration of succinylphosphonate methyl ester (B).

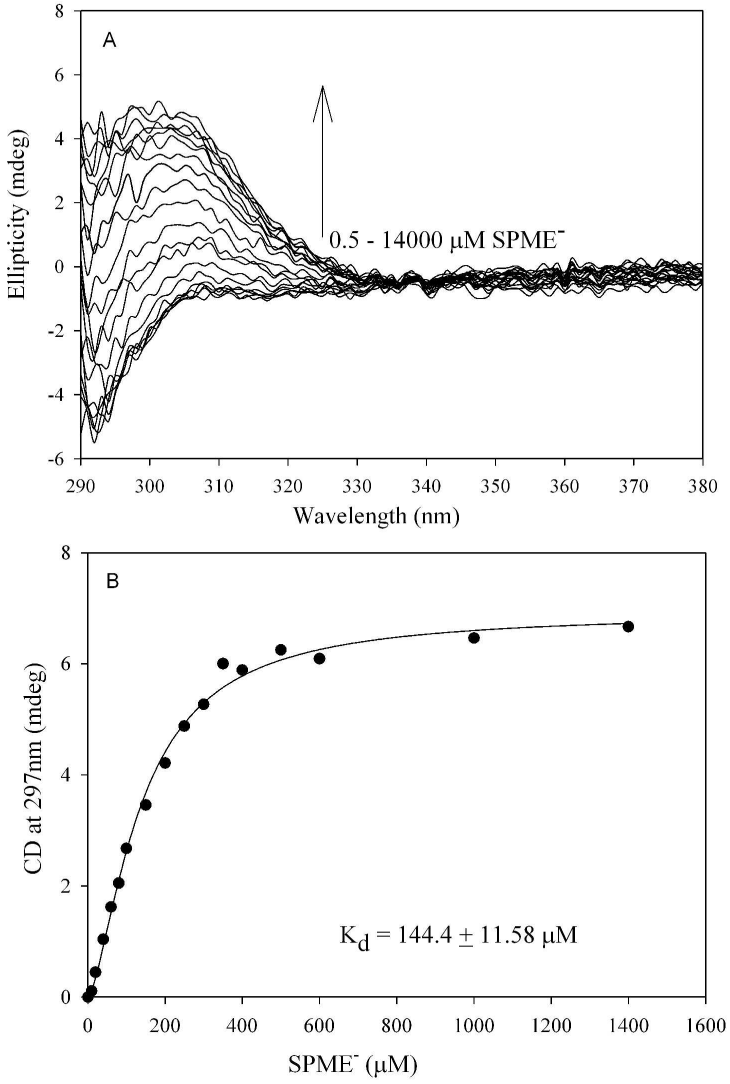


Figure S10. Circular dichroism titration of His298Asp with propionylphosphinate. The H298D E1o (2.5 mg/mL, concentration of active centers=23.8 μM) in 20 mM KH_2PO_4 (pH 7.0) containing 2 mM $MgCl_2$ and 0.2 mM ThDP was titrated by propionylphosphinate (5-580 μM). Dependence of the ellipticity at 300 nm on the concentration of propionylphosphinate.

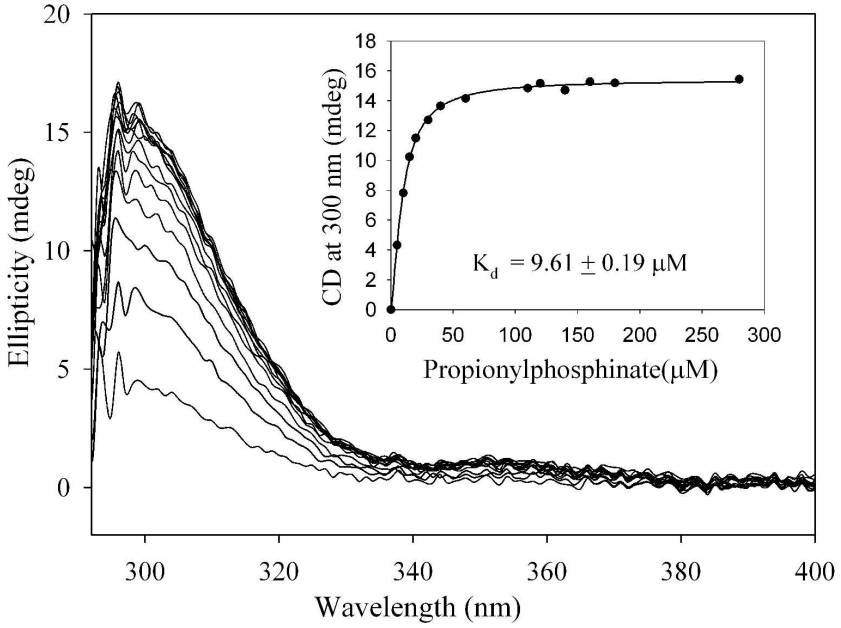


Figure S11. Circular dichroism titration of H298L E1o with propionylphosphinate. Inset: dependence of the ellipticity at 300 nm on concentration of propionylphosphinate.

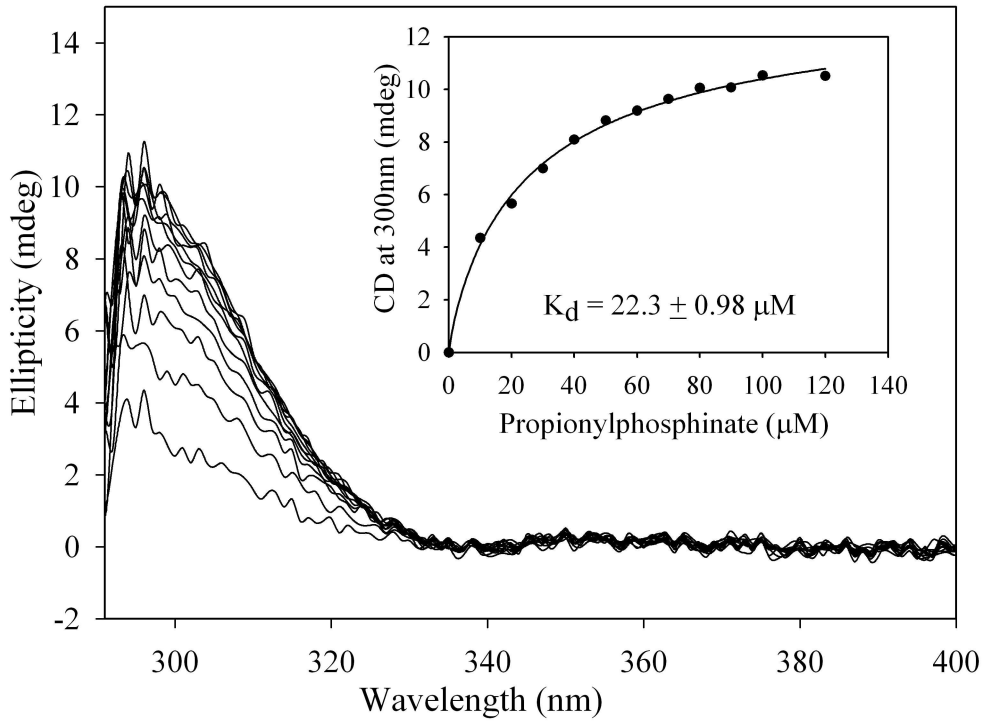


Figure S12. Circular dichroism titration of H298V E1o with propionylphosphinate. Inset: the dependence of the ellipticity at 297 nm on concentration of propionylphosphinate.

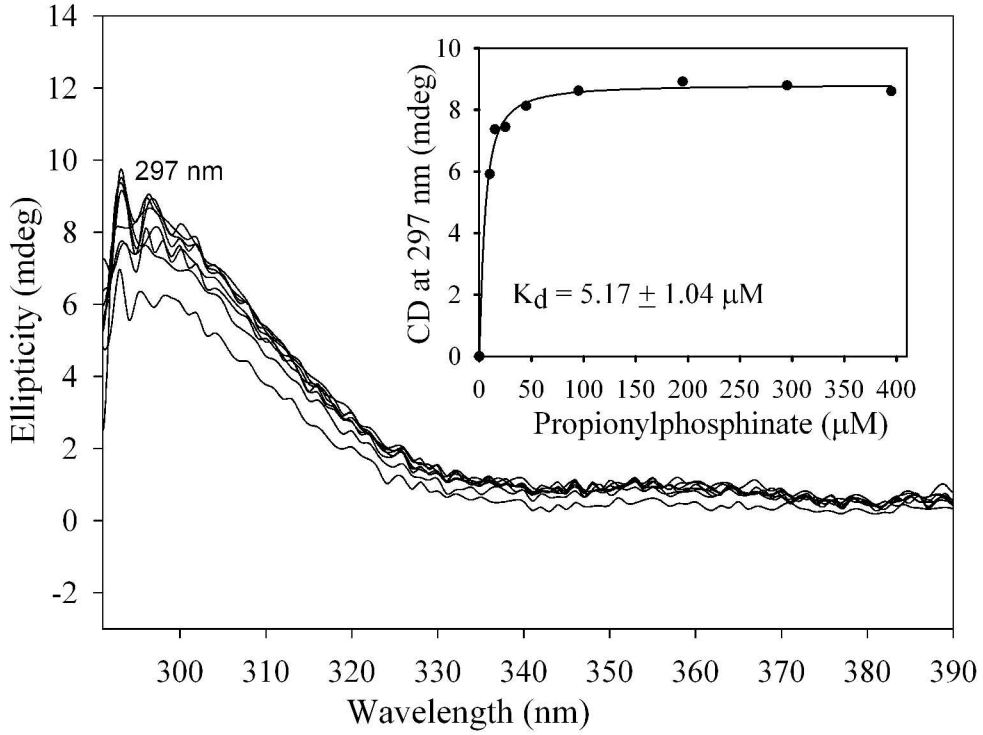


Figure S13. Circular dichroism titration of H298T E1o with propionylphosphinate.

Inset: dependence of the ellipticity at 297 nm on concentration of propionylphosphinate.

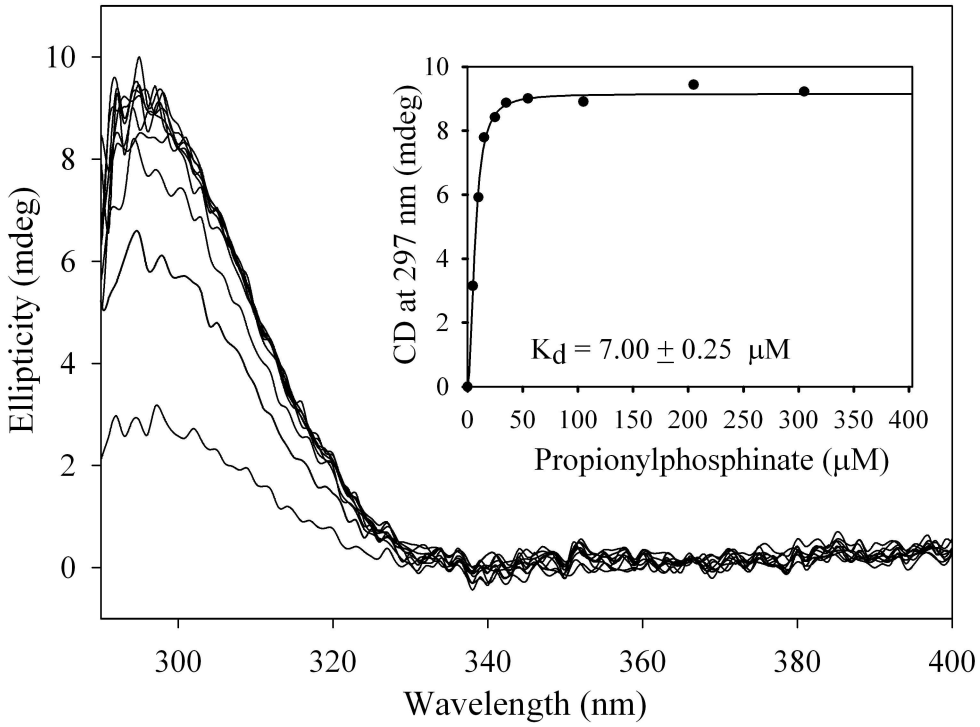


Figure S14. Dependence of ellipticity at 305 nm on concentration