Supplementary Data

The Three-Dimensional Structural Basis of Type II Hyperprolinemia

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Table S1. Kinetic constants for HsP5CDH determined from global fitting

Parameter	Best fit value	Lower Bound	Upper Bound
k_1 (k_{cat}/K_m, NAD)	98.7 $mM^{-1}s^{-1}$	74.7 $mM^{-1}s^{-1}$	144 $mM^{-1}s^{-1}$
k_{-1}	0.473 s^{-1}	0.294 s^{-1}	0.813 s^{-1}
k_2 (k_{cat}/K_m, P5C)	316 $mM^{-1}s^{-1}$	253 $mM^{-1}s^{-1}$	406 $mM^{-1}s^{-1}$
k_{-2}[a]	$\leq$ 300 s^{-1}	-	-
k_3[b]	$\geq$ 500 s^{-1}	-	-
k_{-3}[c]	0	-	-
k_4[b]	$\geq$ 500 s^{-1}	-	-
k_{-4}[d]	0	-	-
k_5 (k_{cat})	10 s^{-1}	9.1 s^{-1}	11 s^{-1}
k_{-5}[e]	0	-	-
k_6[f]	81.7 $mM^{-1}s^{-1}$	-	-
k_{-6}	9.2 s^{-1}	5.1	14.8
$K_I = k_{-6}/k_6$	112 µM	49[g]	181

[a] k_{-2} has little effect on the initial velocity progress curves and was held fixed to a value of 22 s^{-1} but could be $\leq$ 300 s^{-1}.

[b] k_3 and k_4 have little effect on the initial velocity progress curves beyond 500 s^{-1} and were fixed at 3290 s^{-1} and 569s^{-1} so that these steps were non rate limiting according to the Theorell-Chance mechanism.[1]

[c] k_{-3} was fixed to zero based on the previous observation of no observable turnover in the reverse direction.

[d] k_{-4} was fixed to zero according to the Theorell-Chance mechanism.

[e] k_{-5} was fixed to zero based on the observation of a $K_d \geq 1$ mM (data not shown). Once k_{-5} is 10-fold below k_5 it has no effect on the progress curves.

[f] k_6 was held fixed to obtain an error on the K_I; this step is at equilibrium and thus the fixed rate constant shown here is only the best fit value and should not be considered as a constrained estimate of this rate constant.

[g] The percent error from the bounds of k_{-6} was used to estimate a rigorous boundary for the K_I.

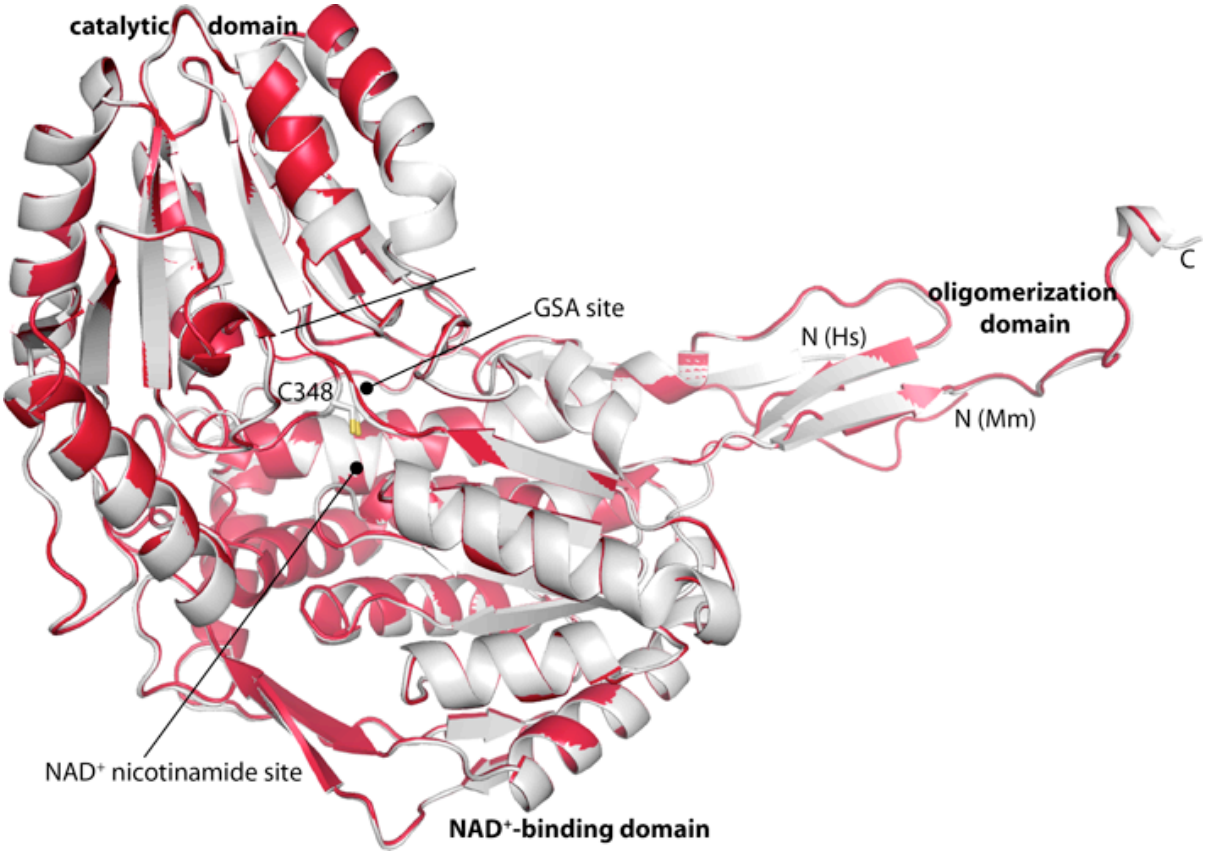

Figure S1. Superposition of HsP5CDH (white) and MmP5CDH (red).

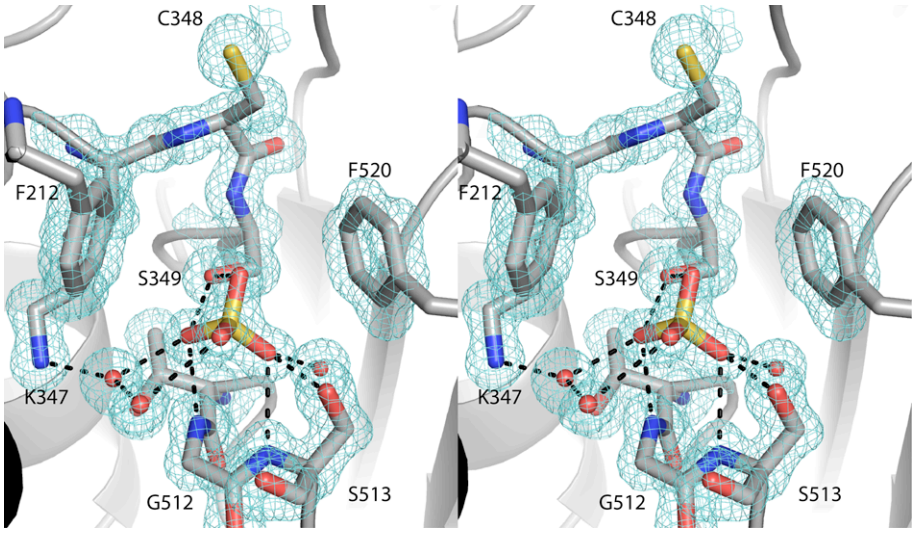

Figure S2. Electron density for the active site of the MmP5CDH-sulfate complex (stereographic view). The cage represents a simulated annealing σ_A-weighted F_o - F_c omit map contoured at 3.0 σ.

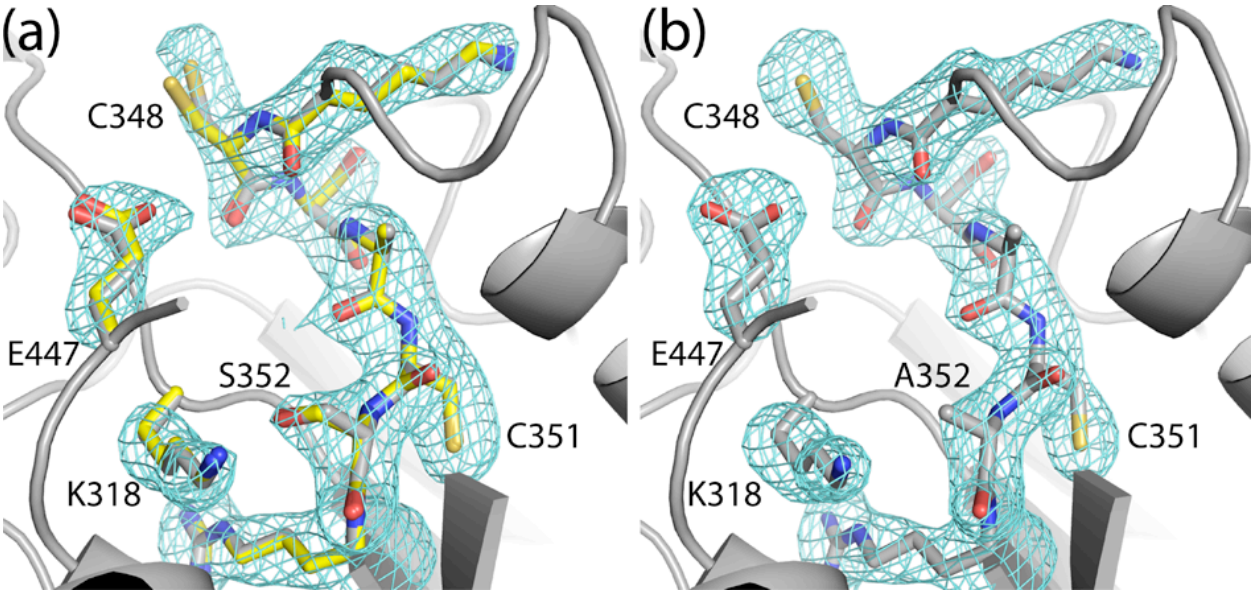

Figure S3. Electron density for the catalytic loops of (a) HsP5CDH and (b) S352A. (a) Superposition of HsP5CDH (gray) and MmP5CDH (yellow). The cage represents a simulated annealing σ_A-weighted F_o - F_c omit map for HsP5CDH contoured at 3.0 σ. Note that the two enzymes have almost identical active site conformations. (b) Catalytic loop of S352A shown in the same orientation as in panel a. The cage represents a simulated annealing σ_A-weighted F_o - F_c omit map contoured at 3.0 σ. Note that the conformation of the active site of S352A is nearly identical to those of HsP5CDH and MmP5CDH.

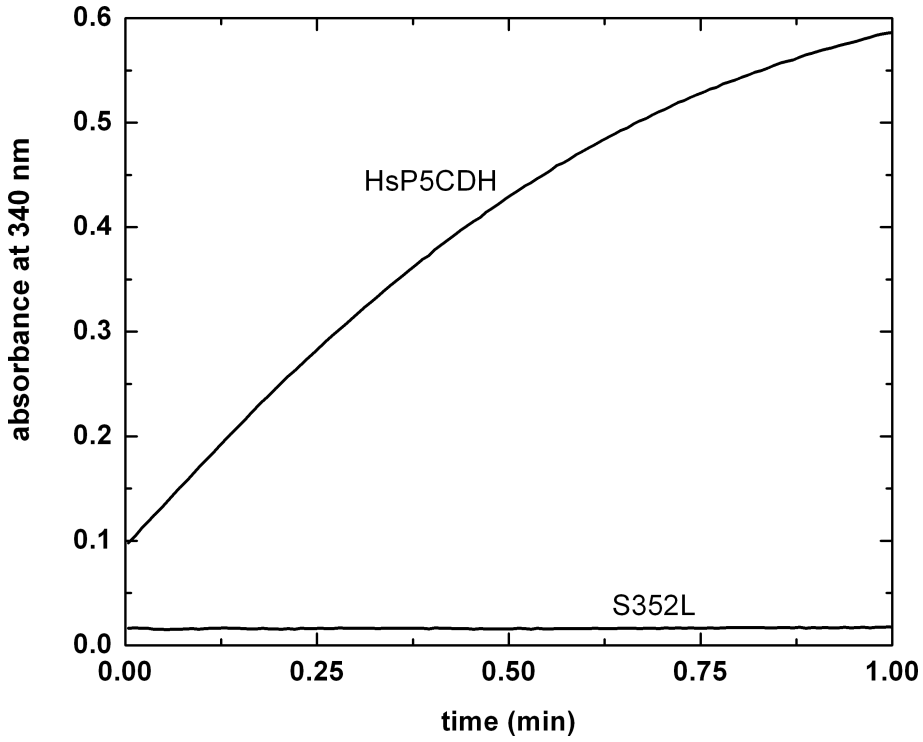

Figure S4. Mutation of Ser352 to Leu abolishes catalytic activity. Progress curves for HsP5CDH and S352L. The NAD^+ and P5C concentrations are 350 μM and 200 μM, respectively. The concentration of S352L is 10-fold higher than that of HsP5CDH.

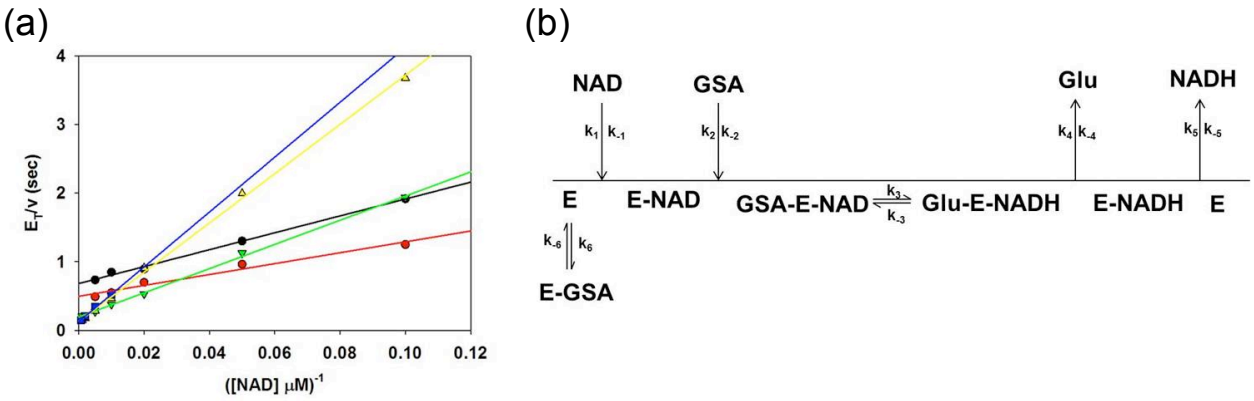

Figure S5. (a) Lineweaver-Burk analysis of initial velocity data for HsP5CDH collected with NAD^+ concentrations in the range 10 - 1500 μM in different fixed concentrations of a 50/50 mixture of DL-P5C with L-P5C concentrations as follows: 10 (black), 15 (red), 50 (green), 150 (yellow), 500 μM (blue). Solid lines are the best-fit line to the individual data sets with the corresponding colored data points. (b) Kinetic scheme suggested by previously published data[2] but with an additional step for substrate inhibition as observed in panel A at higher P5C concentrations (150 and 500 μM). The original kinetic mechanism established for HsP5CDH was a Theorell-Chance mechanism, which is a limiting case of the scheme shown above where k_3 and k_4 are much faster than k_5.

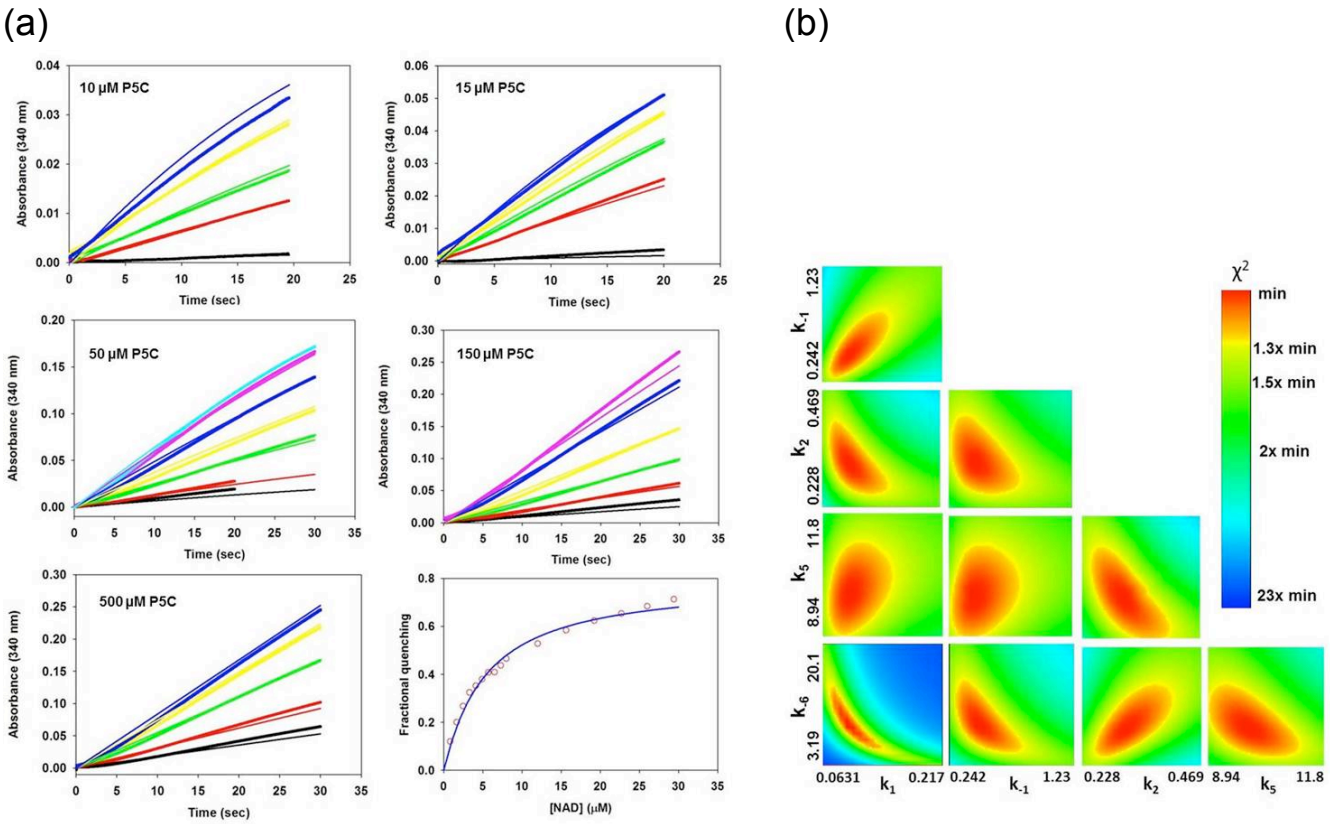

Figure S6. (a) Initial velocity progress curves for HsP5CDH at various NAD^+ concentrations (1-1500 µM) and different fixed L-P5C concentrations followed at 340 nm. Data were globally fitted to the simulated mechanism shown in Supplemental Figure 6B using KinTek Global Kinetic Explorer.[3] The bottom right graph shows a titration of HsP5CDH with NAD^+ monitored by tryptophan fluorescence quenching, which was also included in the global fitting analysis to help constrain k_1 and k_{-1}. The rate constants for the chemical step (k_3) and glutamate dissociation step (k_4) were held fixed at values well above the NADH dissociation step (k_5) in accordance with the Theorell-Chance mechanism.[1] (b) FitSpace[4] contour plots of the global fitting showing how variation in the fitted parameters affects the χ^2 value.

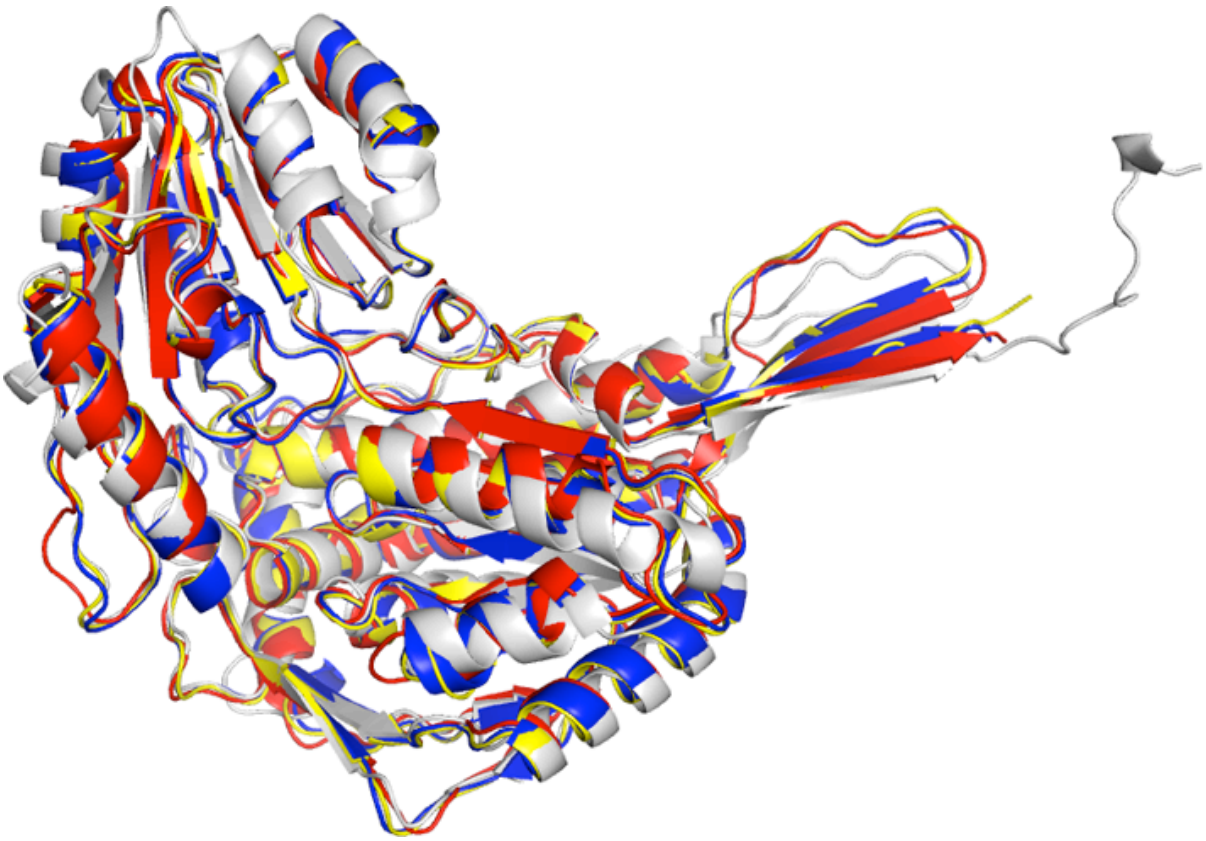

Figure S7. Superposition of HsP5CDH with bacterial P5CDHs from *Thermus thermophilus* (red, PDB code 2EIW), *Bacillus licheniformis* (blue, PDB code 3RJL), and *Bacillus halodurans* (yellow, PDB code 3QAN).

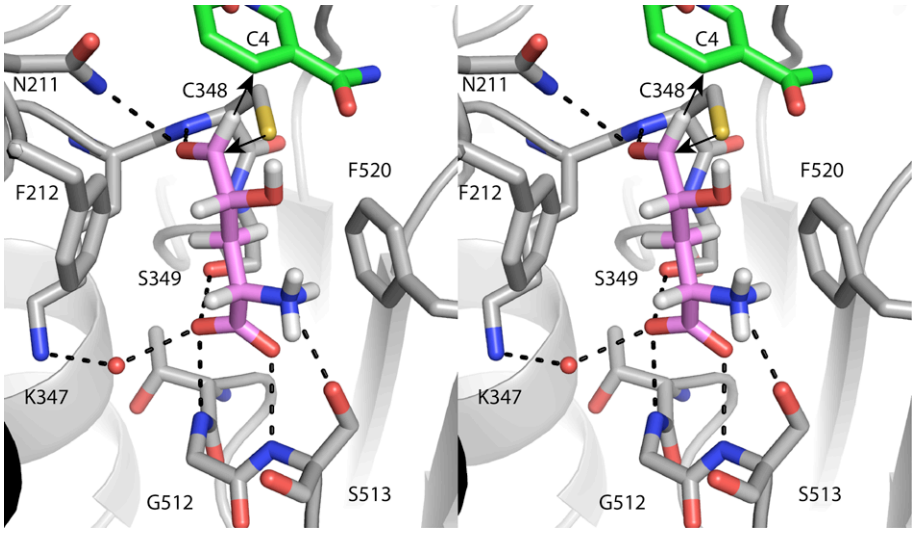

Figure S8. A model of OH-GSA bound to MmP5CDH (stereographic view). OH-GSA is shown in pink. NAD^+ is colored green. The arrows depict the directions of nucleophilic attack by Cys348 and hydride transfer to NAD^+.

Supplementary References

1. Cook, P. F. & Cleland, W. W. (2007). *Enzyme kinetics and mechanism*, Garland Science, New York.
2. Forte-McRobbie, C. & Pietruszko, R. (1989). Human glutamic-gamma-semialdehyde dehydrogenase. Kinetic mechanism. *Biochem. J.* **261**, 935-43.
3. Johnson, K. A., Simpson, Z. B. & Blom, T. (2009). Global kinetic explorer: a new computer program for dynamic simulation and fitting of kinetic data. *Anal. Biochem.* **387**, 20-9.
4. Johnson, K. A., Simpson, Z. B. & Blom, T. (2009). FitSpace explorer: an algorithm to evaluate multidimensional parameter space in fitting kinetic data. *Anal. Biochem.* **387**, 30-41.