SUPPORTING INFORMATION

Mutation of active site glutamate in serine hydroxymethyltransferase allows trapping a reactive intermediate: a combined neutron and X-ray crystallography study

Victoria N. Drago,[1] Robert S. Phillips,[2,3] and Andrey Kovalevsky[1]*

[1]*Neutron Scattering Division, Oak Ridge National Laboratory, Oak Ridge, TN, 37831, USA*

[2]*Department of Chemistry, University of Georgia, Athens, GA, 30602, USA*

[3]*Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, 30602, USA*

* To whom correspondence should be addressed: Andrey Kovalevsky: kovalevskyay@ornl.gov

Table S1. Crystallographic data collection and refinement statistics for the X-ray and neutron structures of *Tth*SHMT E53Q. Values in parentheses are for the highest-resolution shell.

	***Tth*SHMT E53Q** Room Temp. PDB ID 10YN	
Data collection:	**Neutron**	**X-ray**
Beamline/Facility	IMAGINE (HFIR, ORNL)	Rigaku HighFlux HomeLab
Space group	$P2_1$	
Cell dimensions:		
a, *b*, *c* (Å); α, β, γ (°)	58.82, 83.52, 95.43 90.0, 91.6, 90.0	
Resolution (Å)	58.8 – 2.40 (2.52 – 2.40)	95.4 – 1.65 (1.71 – 1.65)
No. reflections measured	97943 (3784)	413563 (25930)
No. reflections unique	28304 (1083)	109873 (10773)
R_{merge}	0.183 (0.301)	0.067 (0.361)
R_{pim}	0.101 (0.171)	0.039 (0.251)
$CC_{1/2}$	0.957 (0.686)	0.967 (0.823)
$\langle I / \sigma I \rangle$	3.8 (2.3)	19.1 (2.5)
Completeness (%)	77.3 (62.4)	99.1 (97.1)
Redundancy	3.5 (3.1)	3.8 (2.4)

Refinement:	**Joint XN**
Resolution (neutron, Å)	40.0 – 2.40
Resolution (X-ray, Å)	40.0 – 1.65
Data rejection criteria	no observation & |F|=0
Sigma cut-off	2.50
No. reflections (neutron)	23605
No. reflections (X-ray)	99395
R_{work} / R_{free} (neutron)	0.236/0.263
R_{work} / R_{free} (X-ray)	0.189/0.197
R_{work} / R_{free} (joint XN)	0.198/0.208
No. atoms	
Protein, including H and D	12563
Sulfate	10
Water	1515 (i.e. 505 D_2O molecules)
B-factors	
Protein	17.4
Sulfate	38.8
Water	40.3
R.M.S. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.10

Table S1 (cont'd).

	***Tth*SHMT E53Q-Ser-THF** Room Temp. PDB ID 10ZB
Data collection:	**X-ray (in-house)**
Diffractometer	Rigaku HighFlux, Eiger R 4M
Space group	$P2_1$
Wavelength (Å)	1.5406
Cell dimensions:	
a, *b*, *c* (Å)	58.9, 83.6, 95.3
α, β, γ (°)	90.0, 91.7, 90.0
Resolution (Å)	95.30 – 1.80 (1.86 – 1.80)
No. reflections unique	85253 (8531)
R_{merge}	0.114 (0.325)
R_{pim}	0.058 (0.171)
$CC_{1/2}$	0.971 (0.870)
$\langle I / \sigma I \rangle$	21.0 (4.6)
Completeness (%)	99.6 (99.6)
Redundancy	5.0 (4.6)
Refinement:	
R_{work} / R_{free}	0.157/0.185
B-factors	
Protein	14.9
PLP-L-Ser	9.1
THF	32.2
Acetate	23.2
Sulfate	52.4
Water	24.5
R.M.S. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.001
All atom clash score	1.97

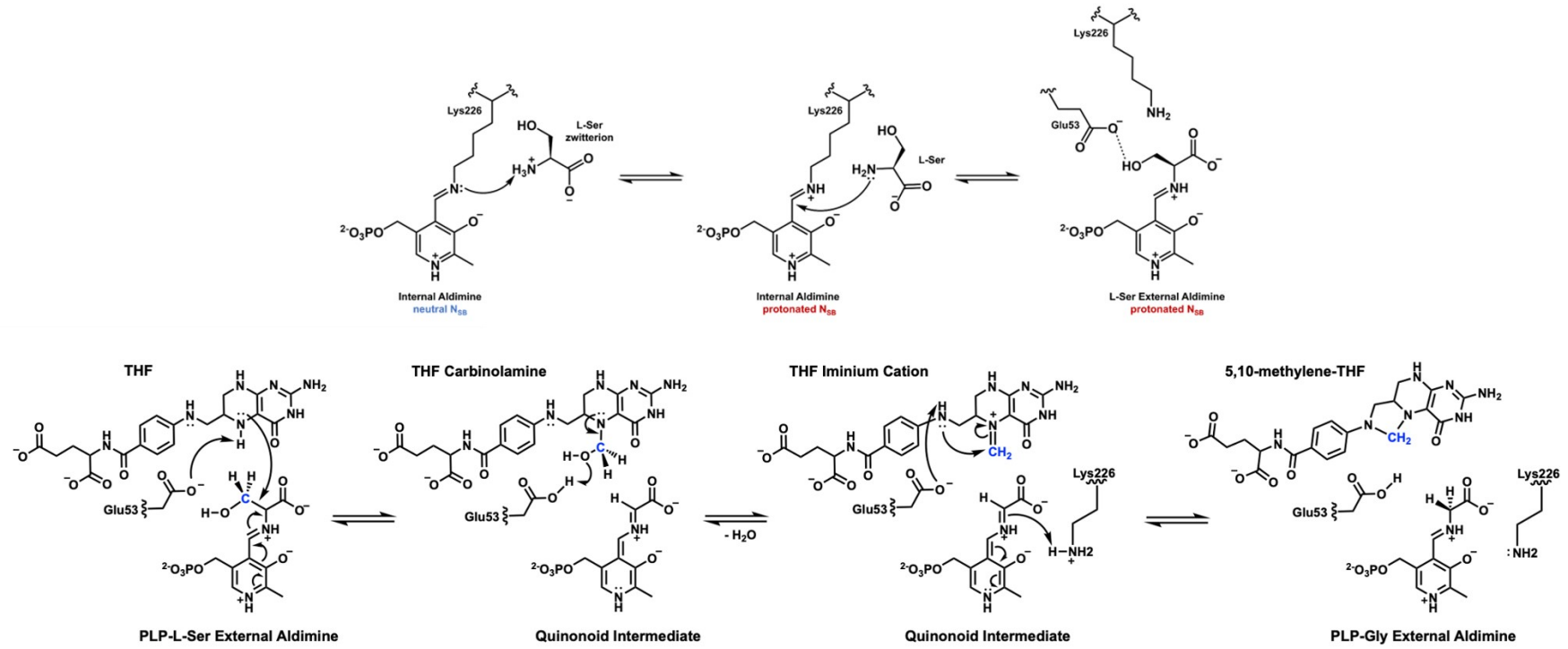

Figure S1. (top) The proposed mechanism for L-Ser attack on the Schiff base of the PLP internal aldimine that ultimately leads to the formation of the PLP-L-Ser external aldimine. (bottom) The proposed direct displacement mechanism for THF-dependent reaction catalyzed universally by the SHMT active site glutamate. The catalytic Lys carries out transformation of the PLP quinonoid intermediate into the PLP-Gly external aldimine (Drago et al. 2023, 2024).

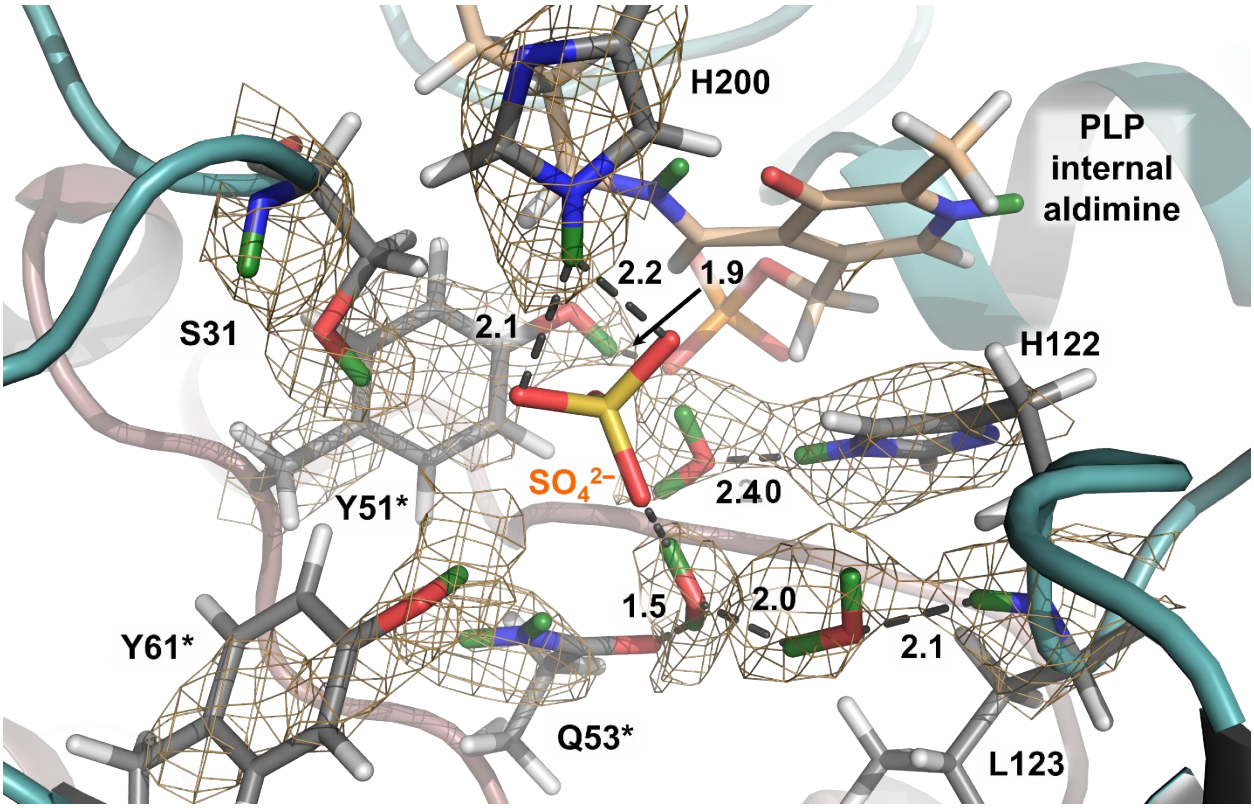

Figure S2. The $2F_O–F_C$ neutron scattering length density map for the active site in *Tth*SHMT E53Q, shown as light brown mesh, is contoured at 1.2 σ level. The figure highlights several D_2O water molecules and residues with partially H/D exchanged hydroxyl groups, including S31, Y51*, Q53, and Y61*. H atoms are colored white, and D atoms are green. Distances are in Angstroms.

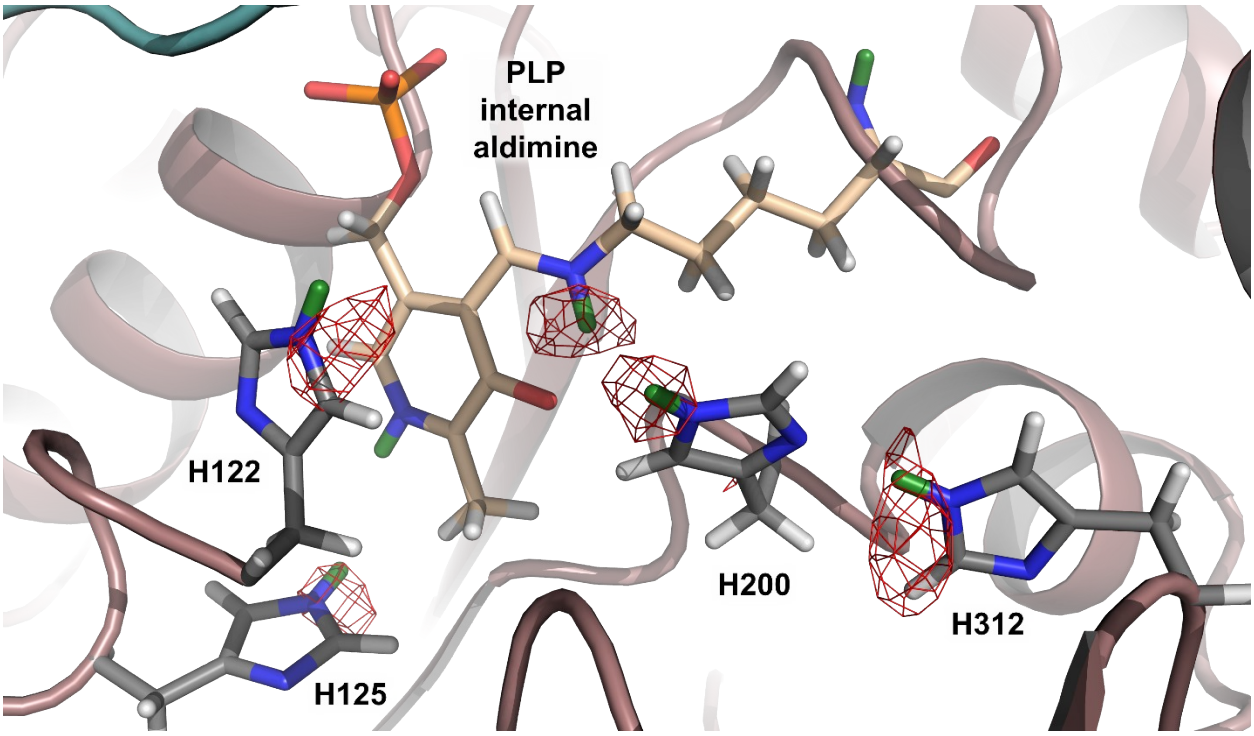

Figure S3. The F_O–F_C omit difference map for the His and PLP internal aldimine Schiff base D atoms contoured at 2.5 σ level is shown in red mesh (protomer B). H atoms are colored white, and D atoms are green.

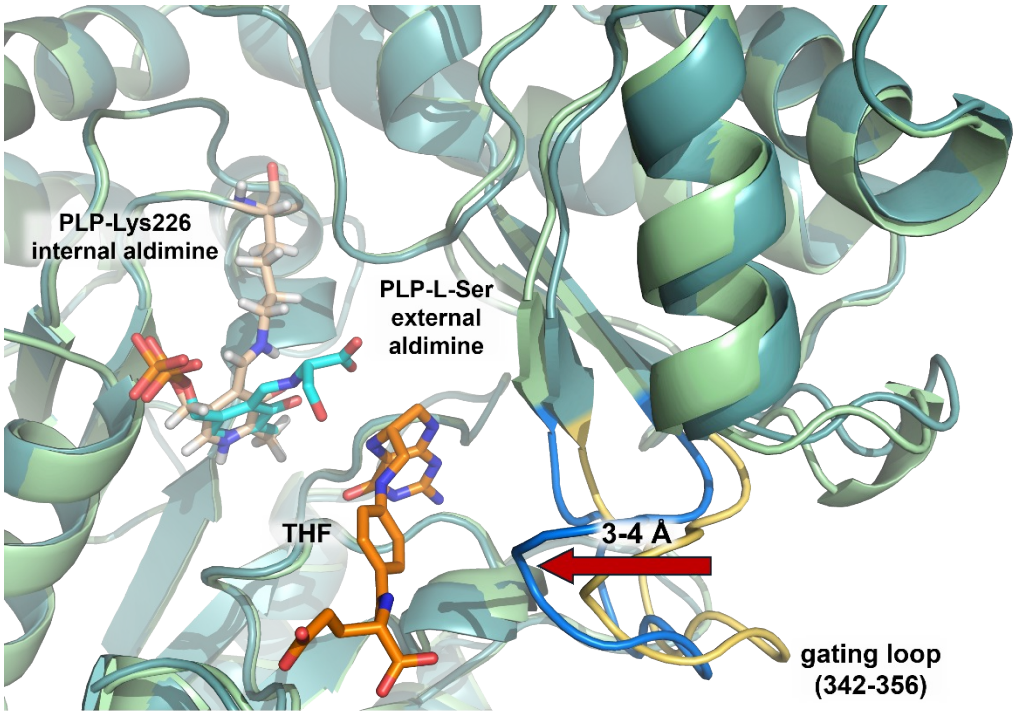

Figure S4. Superposition of E53Q holoenzyme (protomer A, cartoon colored in light teal) and E53Q-Ser-THF complex (protomer B, cartoon colored pale green) highlighting the locations of PLP internal aldimine, PLP-L-Ser external aldimine, THF and the gating loop motion upon THF binding from open conformation (colored light orange) to closed conformation (colored light blue). All atoms are colored by atom type.

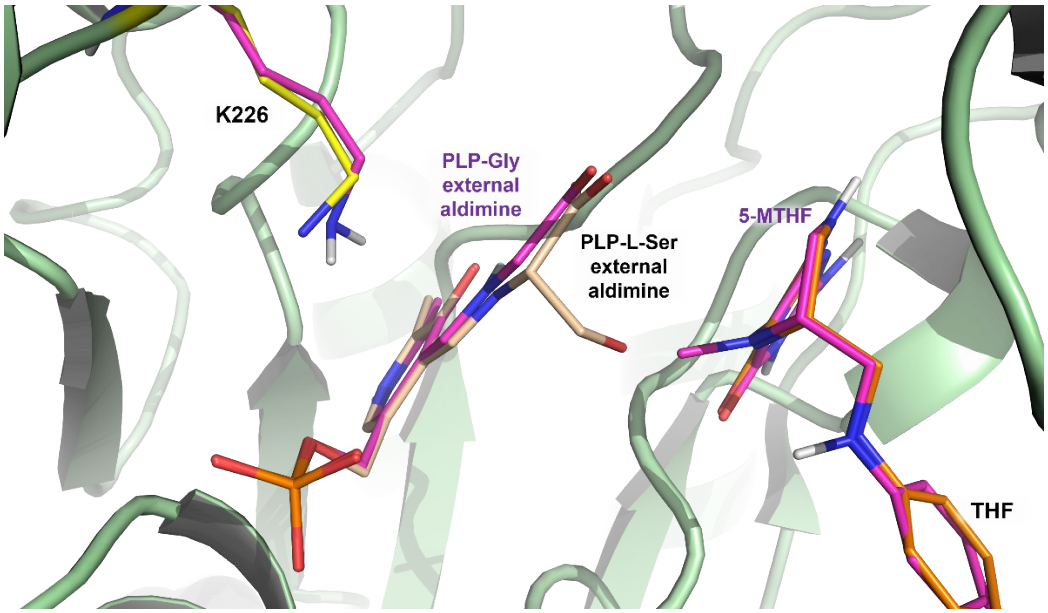

Figure S5. Superposition of E53Q-Ser-THF (protomer B, cartoon colored in pale green) and Gly-5MTHF complex (protomer B, cartoon not shown, PDB ID 5O5G) highlighting the locations of PLP-L-Ser and PLP-Gly external aldimines, THF, 5-MTHF and K226. All atoms are colored by atom type. Carbon atoms in Gly-5MTHF complex are colored magenta. For E53Q-Ser-THF, carbon atoms are yellow for K226, beige for PLP-L-Ser, and orange for THF.

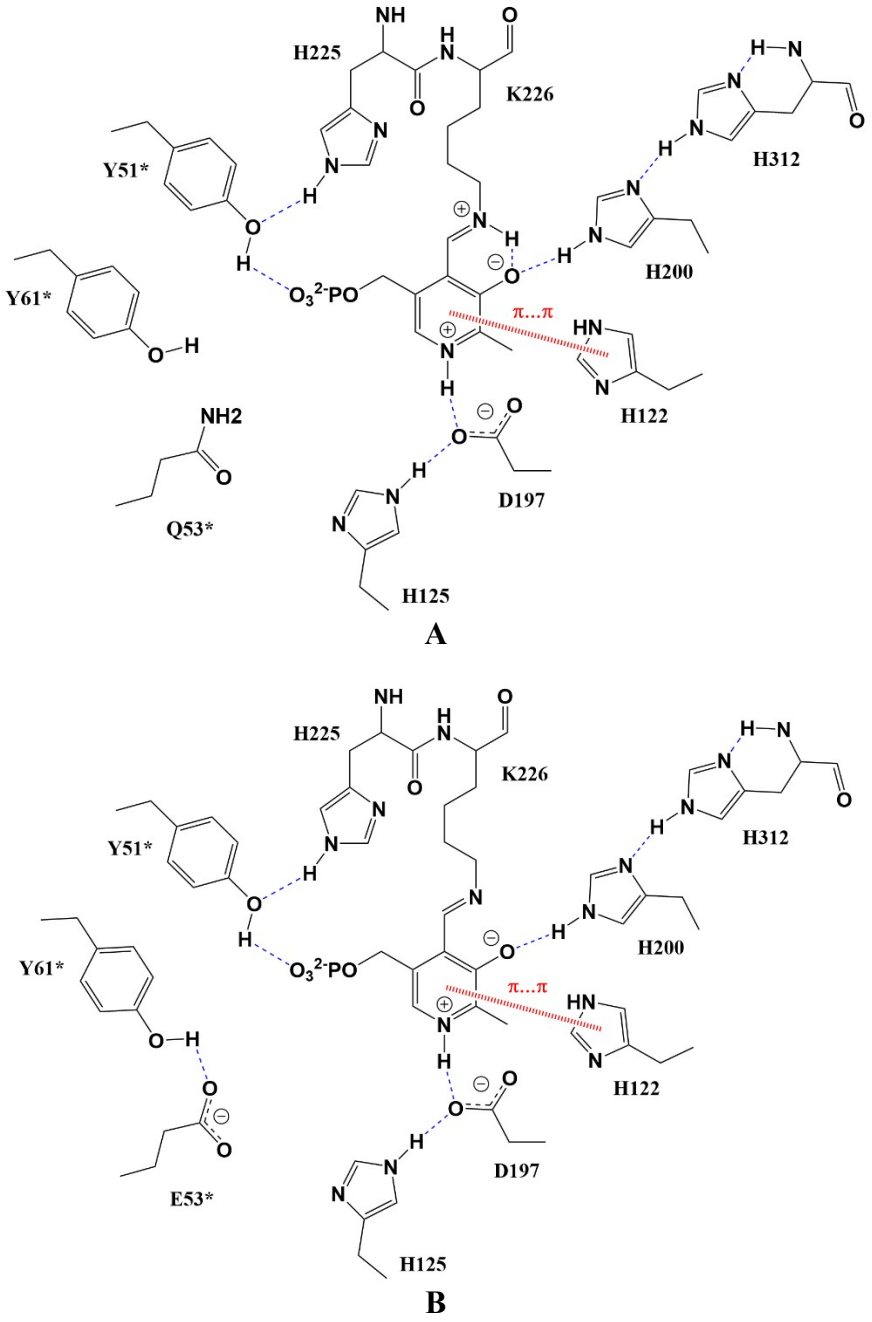

Figure S6. Chemical diagrams of the TthSHMT active site depicting the protonation states and electrical charges determined by neutron crystallography in (**A**) E53Q mutant (this work), and (**B**) wild-type enzyme (PDB ID 8SUJ, Drago et al. 2023). Hydrogen bonds are shown by blue dashed lines.

References

Drago VN, Campos C, Hooper M, Collins A, Gerlits O, Weiss KL, Blakeley MP, Phillips RS & Kovalevsky A (2023) Revealing protonation states and tracking substrate in serine hydroxymethyltransferase with room-temperature X-ray and neutron crystallography. *Commun Chem* 6, 162.

Drago VN, Phillips RS & Kovalevsky A (2024) Universality of critical active site glutamate as an acid-base catalyst in serine hydroxymethyltransferase function. *Chem Sci* 15, 12827-12844.