SUPPORTING INFORMATION

Universality of critical active site glutamate as acid-base catalyst in serine hydroxymethyltransferase function

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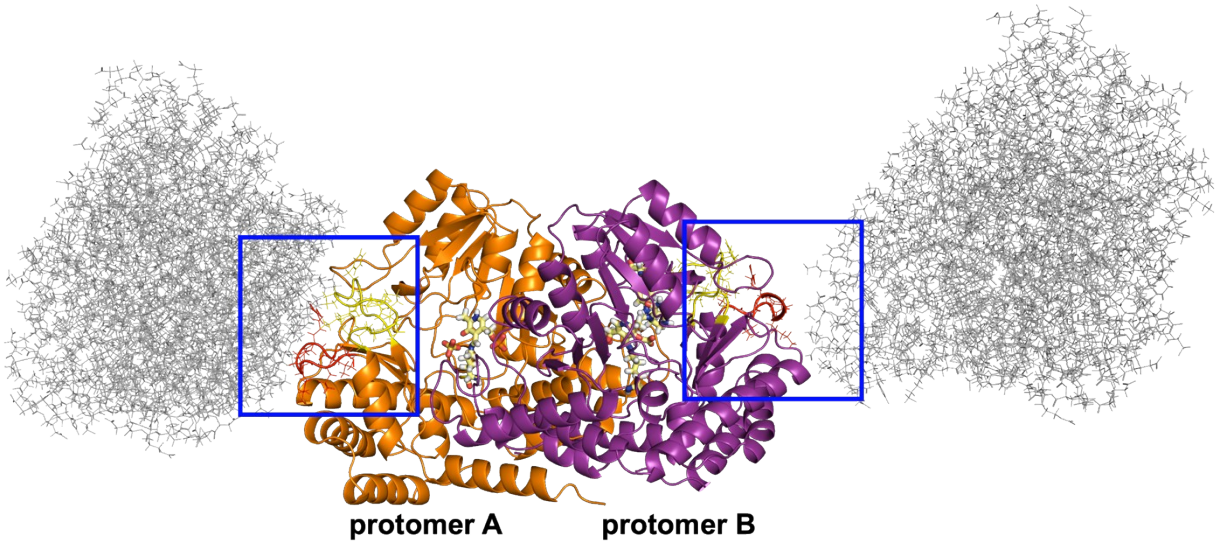
Table S1. Crystallographic data collection and refinement statistics for the X-ray and neutron structures of *Tth*SHMT and hSHMT2. Values in parentheses are for the highest-resolution shell.

	***Tth*SHMT/FA** Room Temp. PDB ID 9BPE	
Data collection:	**Neutron**	**X-ray**
Beamline/Facility	MaNDi (SNS, ORNL)	Rigaku HighFlux HomeLab
Space group	$P2_1$	
Cell dimensions:		
a, *b*, *c* (Å); α, β, γ (°)	58.8, 83.5, 95.6; 90.0, 91.5, 90.0	
Resolution (Å)	14.5 – 2.30 (2.37-2.3)	95.5 – 2.0 (2.07 – 2.0)
No. reflections measured	157996 (12103)	229686 (22081)
No. reflections unique	38362 (3307)	62375 (6207)
R_{merge}	0.216 (0.266)	0.124 (0.462)
R_{pim}	0.109 (0.141)	0.074 (0.279)
$CC_{1/2}$	0.911 (0.437)	0.987 (0.730)
$\langle I / \sigma I \rangle$	7.3 (3.2)	9.4 (2.2)
Completeness (%)	92.5 (80.1)	99.9 (99.3)
Redundancy	4.1 (3.7)	3.7 (3.6)

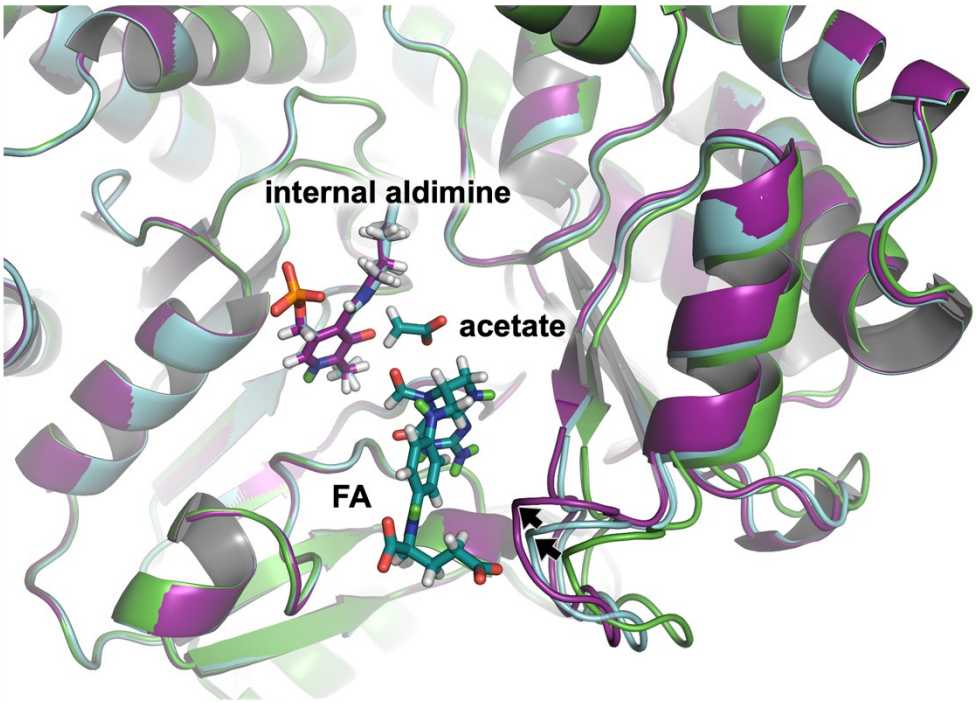
Refinement:	**Joint XN**
Resolution (neutron, Å)	40.0-2.30
Resolution (X-ray, Å)	40.0-2.00
Data rejection criteria	no observation & |F|=0
Sigma cut-off	2.50
No. reflections (neutron)	38074
No. reflections (X-ray)	55831
R_{work} / R_{free} (neutron)	0.212/0.239
R_{work} / R_{free} (X-ray)	0.167/0.187
R_{work} / R_{free} (joint XN)	0.185/0.209
No. atoms	
Protein, including H and D	12567
FA	55
Acetate	7
Sulfate	5
Water	1311 (i.e. 437 D_2O molecules)
B-factors	
Protein	18.5
FA	18.8
ACT	12.6
Sulfate	47.0
Water	42.2
R.M.S. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.01

Table S1 (cont'd).

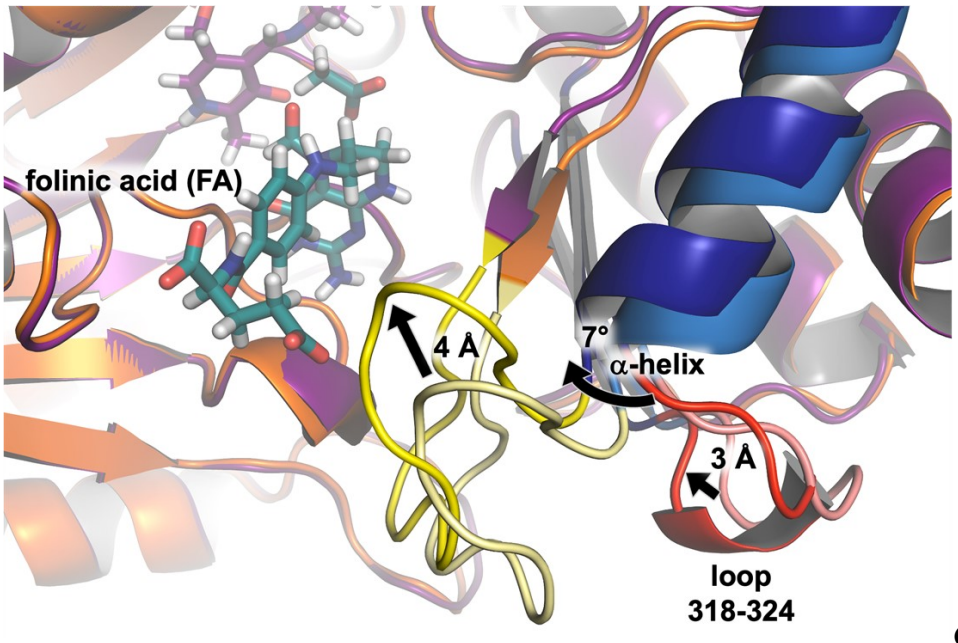
	***Tth*SHMT-Gly/FA** Room Temp. PDB ID 9BOH	***Tth*SHMT-L-Ser/FA** 100K PDB ID 9BOW	**hSHMT2-Gly/FA** Room Temp. PDB ID 9BOX
Data collection:	**X-ray (in-house)**	**X-ray (in-house)**	**X-ray (synchrotron)**
Diffractometer	Rigaku HighFlux, Eiger R 4M	Rigaku HighFlux, Eiger R 4M	ID-19, Advanced Photon Source
Space group	$P2_1$	$P2_1$	$P222_1$
Wavelength (Å)	1.5406	1.5406	0.979
Cell dimensions:			
a, *b*, *c* (Å)	58.8, 83.3, 95.5	58.1, 82.8, 94.4	143.0, 119.3, 134.6
α, β, γ (°)	90.0, 91.7, 90.0	90.0, 91.6, 90.0	90.0, 90.0, 90.0
Resolution (Å)	29.74 – 2.00 (2.05 – 2.00)	29.05 – 1.80 (1.84 – 1.80)	50.00 – 2.10 (2.14 – 2.10)
No. reflections unique	61808 (4473)	76390 (2744)	125654 (6402)
R_{merge}	0.035 (0.085)	0.030 (0.111)	0.113 (0.739)
R_{pim}	0.024 (0.059)	0.026 (0.105)	0.062 (0.427)
$CC_{1/2}$	0.998 (0.993)	0.997 (0.969)	0.987 (0.483)
$\langle I / \sigma I \rangle$	27.9 (15.1)	23.3 (7.6)	12.9 (2.2)
Completeness (%)	99.1 (97.5)	92.2 (56.4)	93.4 (95.7)
Redundancy	5.6 (5.4)	3.5 (2.2)	4.4 (4.1)
Refinement:			
R_{work} / R_{free}	0.131/0.155	0.132/0.159	0.162/0.189
B-factors			
Protein	19.1	11.7	26.6
FA	21.7	20.1	23.5
PLP-Gly	18.1	---	15.6
PLP-L-Ser	---	12.9	---
Sulfate	35.4	49.0	---
Water	29.4	22.9	29.65
R.M.S. deviations			
Bond lengths (Å)	0.015	0.014	0.009
Bond angles (°)	1.364	1.24	0.973
All atom clash score	1.97	1.73	3.05



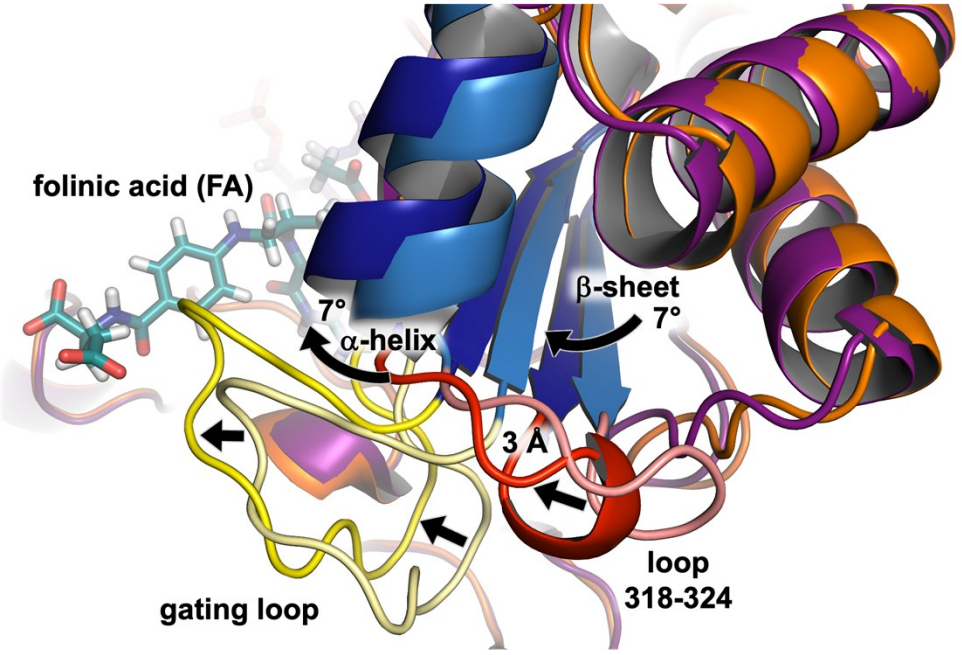
A



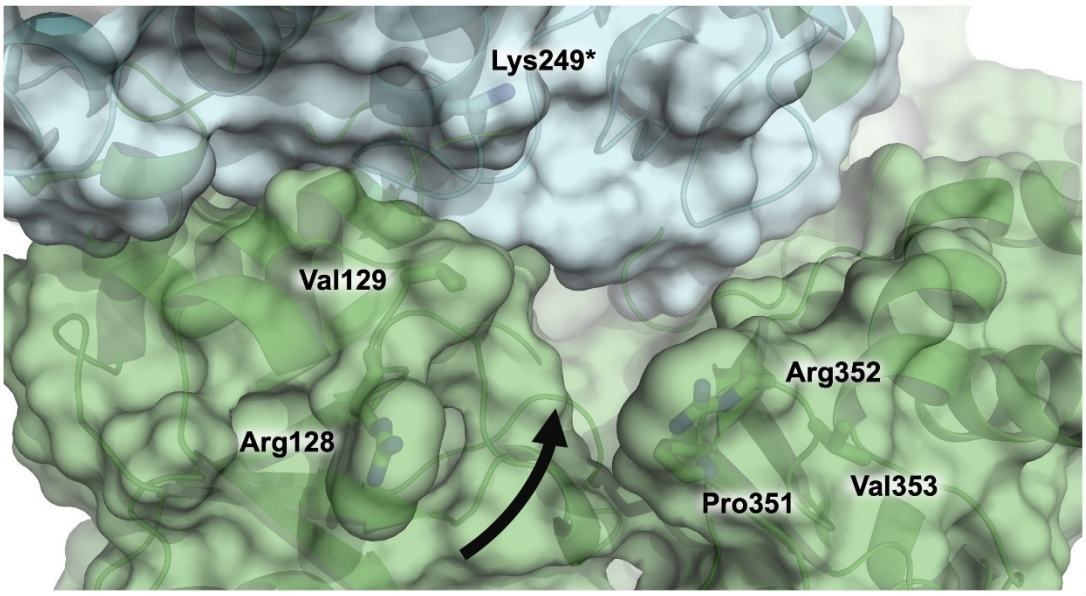
B


folinic acid (FA)
4 Å
7°
α-helix
3 Å
loop
318-324

C


folinic acid (FA)
7°
α-helix
β-sheet
7°
3 Å
loop
318-324
gating loop

D



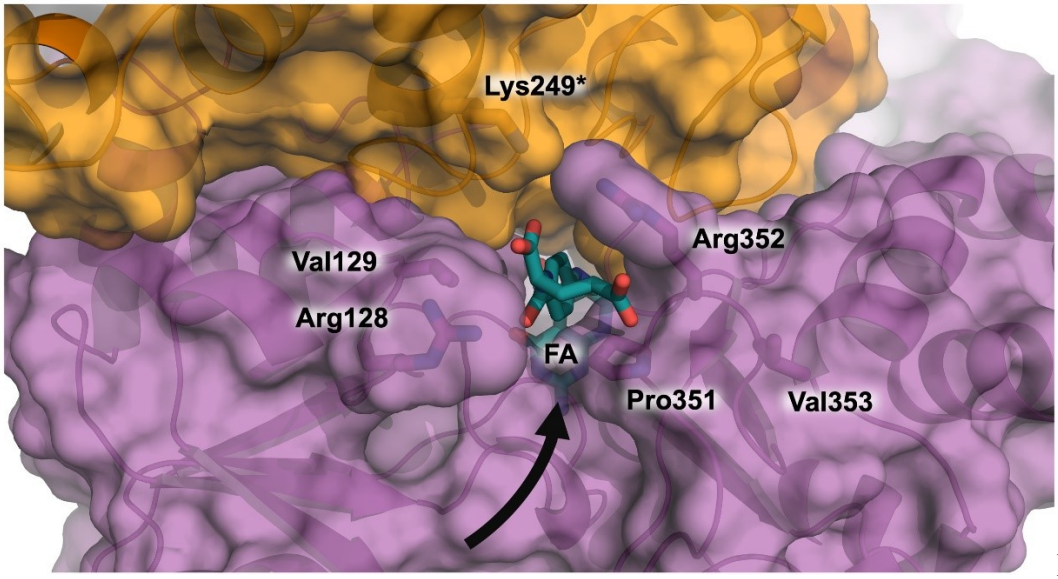


Figure S1. a) *Tth*SHMT crystal packing. Protomer A (orange cartoon) has the nearby symmetry-related molecule (light grey) that is close to the gating loop (yellow) and the loop containing residues 318-324 (red), possibly preventing its active site from being reactive towards amino acid substrates and able to bind THF analogs. b) Comparison of the rearrangement of the gating loop conformations and flanking structural elements between protomer B in the *Tth*SHMT/FA (magenta) neutron structure and protomers A (green) and B (cyan) in the *Tth*SHMT holoenzyme neutron structure. In protomer A of the *Tth*SHMT holoenzyme the gating loop is open, whereas in protomer B of the same structure the gating loop adopts a partially closed conformation. FA (teal) binding in protomer B of *Tth*SHMT/FA induces the gating loop and adjacent structural features to close, securing the FA in its binding pocket. c) A detailed overview of the structural rearrangements upon FA (teal) binding in *Tth*SHMT/FA protomers A (orange) and B (purple). The gating loop (bright yellow in protomer B, light yellow in protomer A) shifts 4 Å towards the FA

and causes the neighboring loop made of residues 318-324 (red in protomer B, pink in protomer A) to follow, moving 3 Å. d) A different view of the same structural rearrangements in protomers A and B of *Tth*SHMT/FA. The adjacent α-helix and β-sheet (dark blue in protomer B, light blue in protomer A) both tilt by ~7 °. e) Surface representation of FA peripheral binding site in the absence of FA binding where the gating loop (residues 342-356) adopts an open conformation and leaves the active site exposed to solvent. f) Surface representation of FA peripheral binding site in the presence of FA where the gating loop is observed to close as shown with the structural rearrangement of Arg352 and Pro351. Opposite the gating loop, the side chain of Arg128 moves to assist in securing FA in the peripheral binding site.

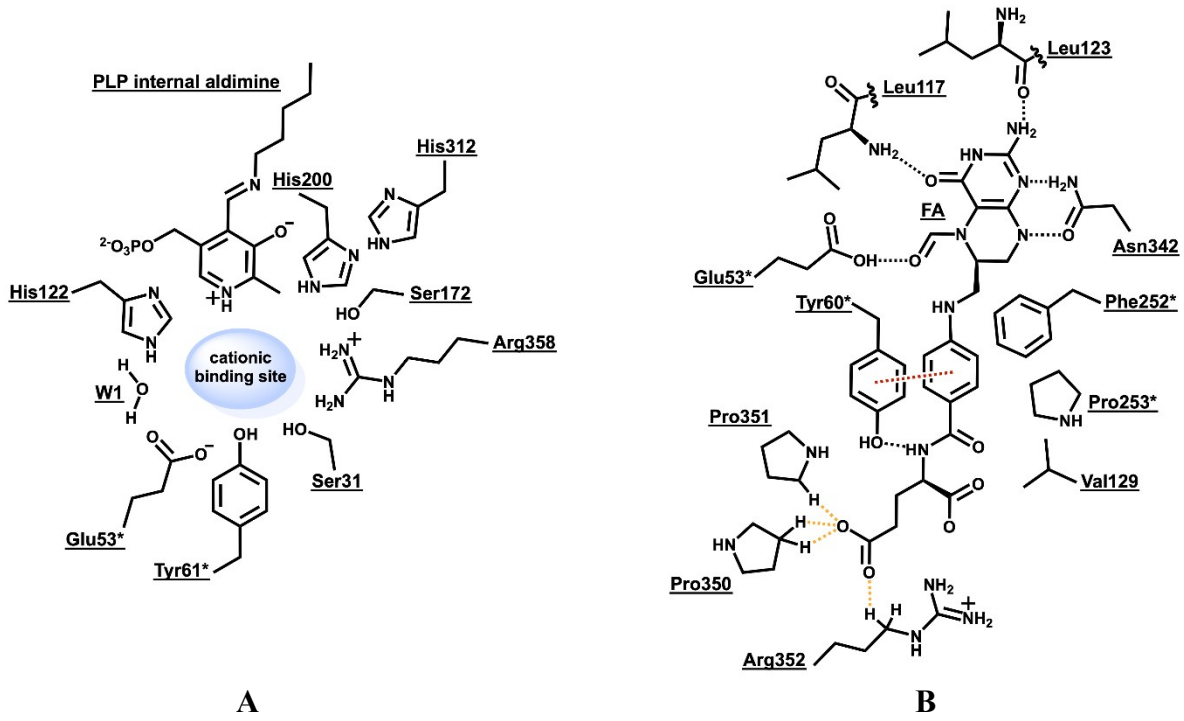


Figure S2. 2D schematics of the amino acid cationic binding site and peripheral FA binding site. a) The cationic amino acid binding site is made up of Arg358, His200, Ser30, Ser172, Tyr61*, Glu53* and His122, which coordinates an active site water, and has an affinity for binding negatively bound molecules. The carboxylate groups of L-Ser and Gly, as well as sulfate ions and acetate molecules, have demonstrated to make a characteristic salt bridge with Arg258. b) FA binds in a pocket adjacent to the active site. H bonding stabilizes the pterin ring at the active site interface while a hydrophobic pocket composed of Tyr60*, Phe252*, Pro253*, Leu117, Leu123, and Pro351. The 4-aminobenzoyl group of THF participates in π-π stacking with Tyr60* and gating loop residues Pro350, Pro351, and Arg352 form C-H···O bonds with its glutamate tail.

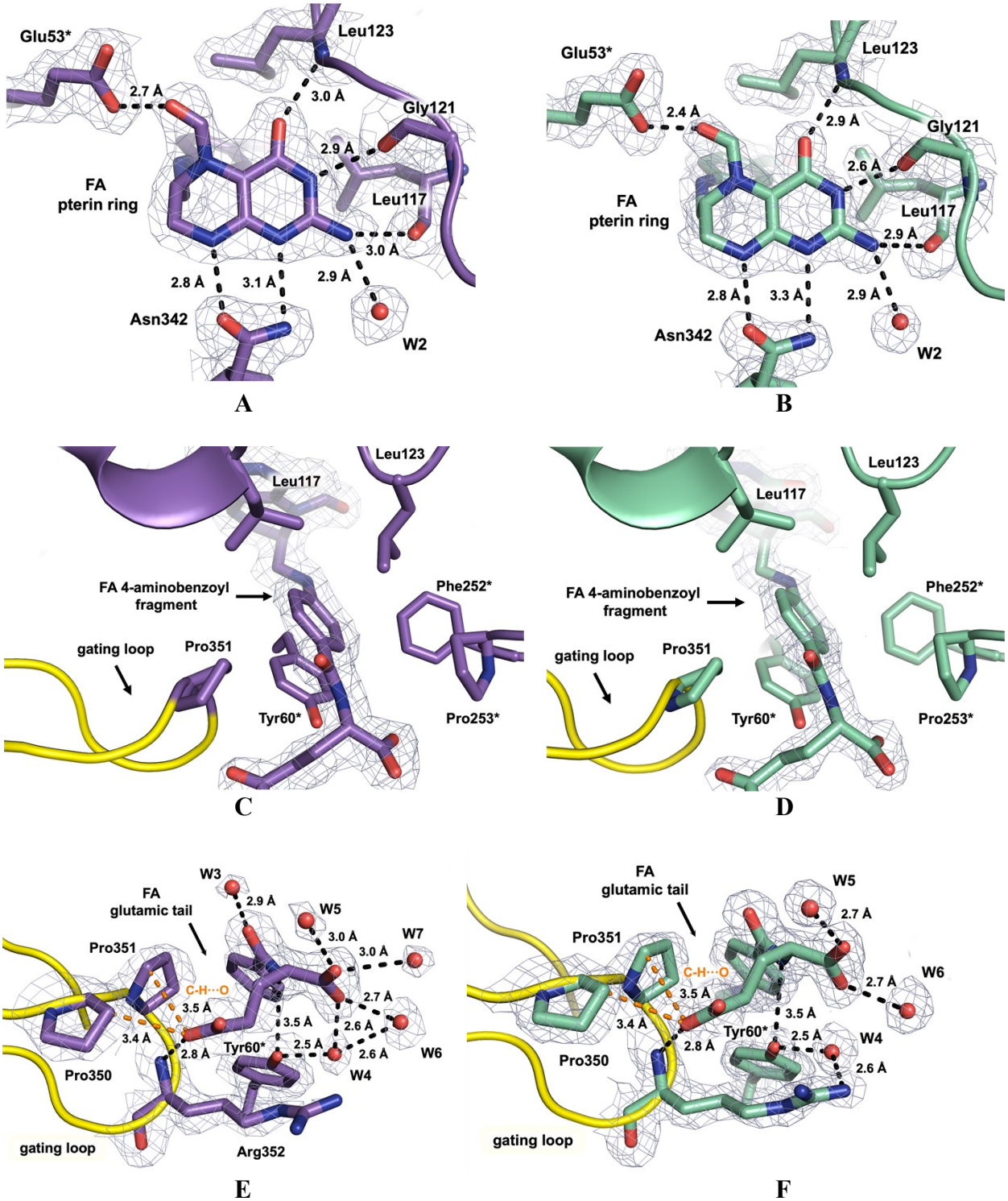
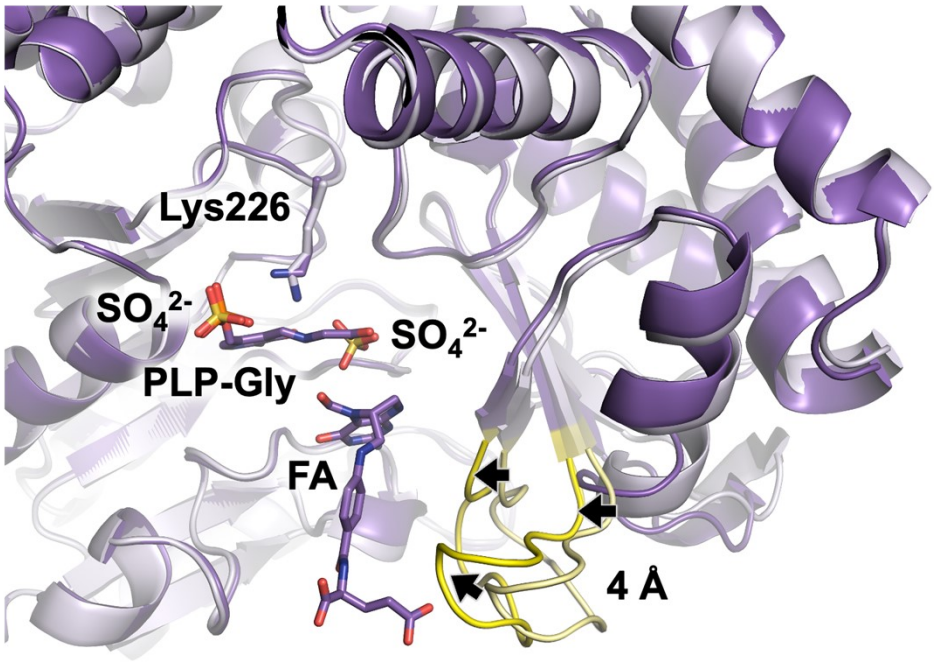
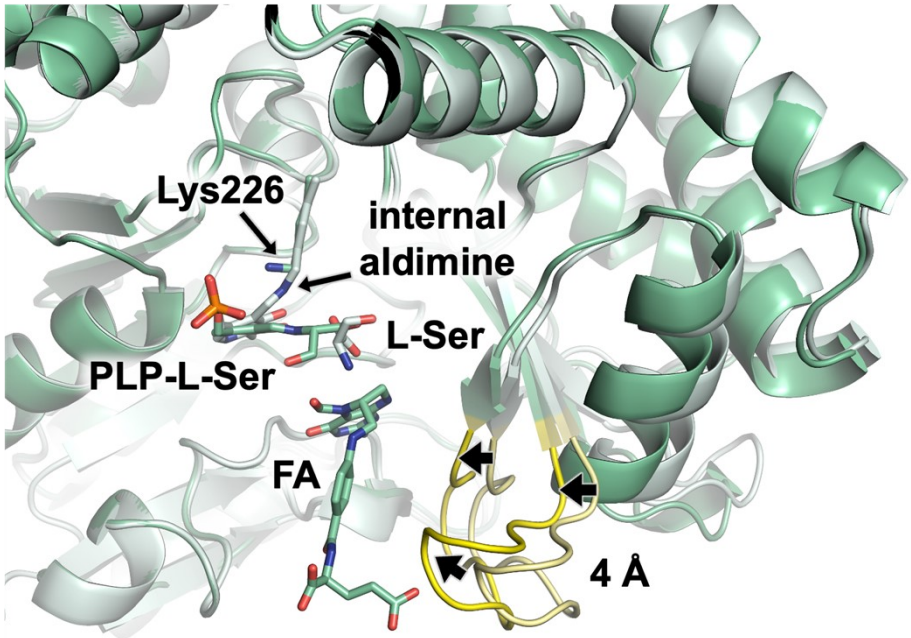


Figure S3. Binding of FA in the *Tth*SHMT-Gly/FA (purple) and *Tth*SHMT-L-Ser/FA (green) ternary complexes. The FA pterin ring blocks the cationic substrate binding site and makes H bonds with Leu123, Gly121, Leu117, Asn342, Glu53*, and a water molecule in *Tth*SHMT-Gly/FA (a) and *Tth*SHMT-L-Ser/FA (b). In both the *Tth*SHMT-Gly/FA (c) and *Tth*SHMT-L-Ser/FA (d) ternary complexes, the 4-aminobenzoyl fragment of FA binds in the hydrophobic cage created by

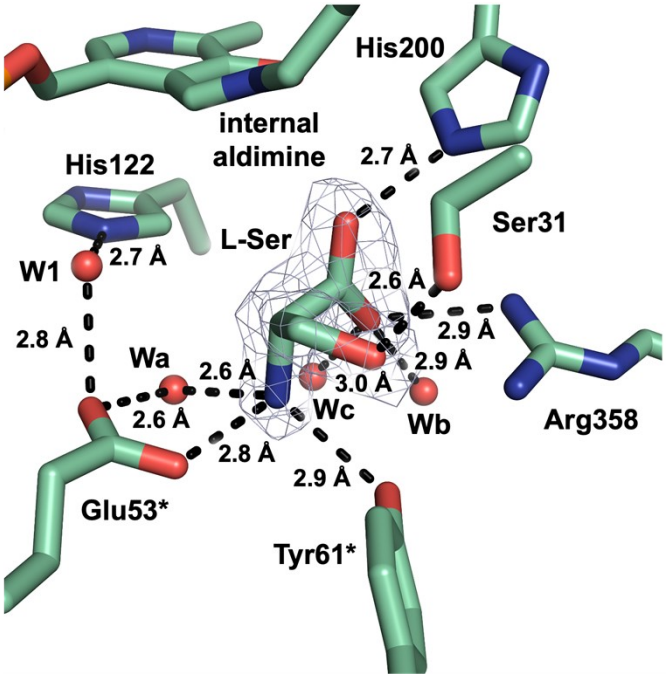
the side chains of Leu117, Leu123, Phe252*, Phe253*, and Pro351. The FA glutamate tail reaches the bulk solvent, with 4 surface water molecules in the *Tth*SHMT-Gly/FA structure (e) and two water molecules in the structure of *Tth*SHMT-L-Ser/FA (f) shielding the negative charge on the main chain carboxylate. In both structures, the side chain glutamate is stabilized through contacts with the gating loop, making C-H□□□O bonds with Pro350 and Pro351 and an H bond with the backbone amide of Arg352. $2F_O$-F_C electron density maps are contoured at 1σ level in all panels.


Lys226
SO_4^{2-}
SO_4^{2-}
PLP-Gly
FA
4 Å

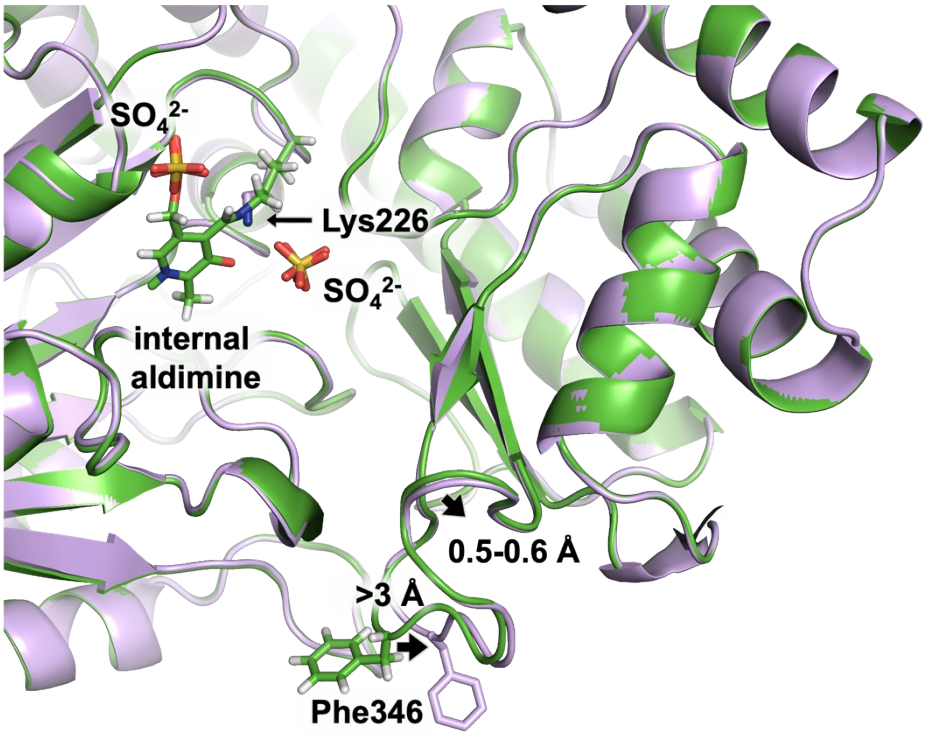
A


Lys226
internal
aldimine
L-Ser
PLP-L-Ser
FA
4 Å

B



C



D

Figure S4. a) Superposition of protomers A (light purple) and B (purple) from the *Tth*SHMT-Gly/FA ternary complex. In protomer B, Gly reacts with PLP to produce the PLP-Gly external aldimine and FA is bound at the peripheral binding site. Protomer A instead lost the PLP coenzyme, and two sulfate ions occupy the active site, residing in the binding locations for the PLP phosphate group and the cationic binding site. The gating loop in protomer B closes by 4 Å compared to protomer A which stays in an open conformation. b) Superposition of protomers A

(light green) and B (green) from the *Tth*SHMT-L-Ser/FA ternary complex. In protomer B, L-Ser reacts with PLP to form the PLP-L-Ser external aldimine and FA binds at the peripheral binding site. In protomer A the PLP internal aldimine is retained and unreacted L-Ser is bound in the cationic binding site. The *Tth*SHMT-L-Ser/FA protomer A gating loop (light yellow) is in an open state and FA binding in protomer B drives its gating loop (bright yellow) to move 4 Å into a closed conformation. c) Binding of the L-Ser molecule in protomer A of *Tth*SHMT-L-Ser/FA at the cationic binding site, showing H bonds as black dashed lines. $2F_O$-F_C electron density map is contoured at 1σ level. d) Superposition of protomers A from the *Tth*SHMT-Gly/FA structure (light purple) and the *Tth*SHMT holoenzyme (bright green). The gating loop is open further when the active site lacks PLP shown by the movement of Phe346 by >3 Å away from the entrance to the active site, possibly as a result of increased flexibility of the loop (residues 116-123) on the opposite side of the active site entrance.

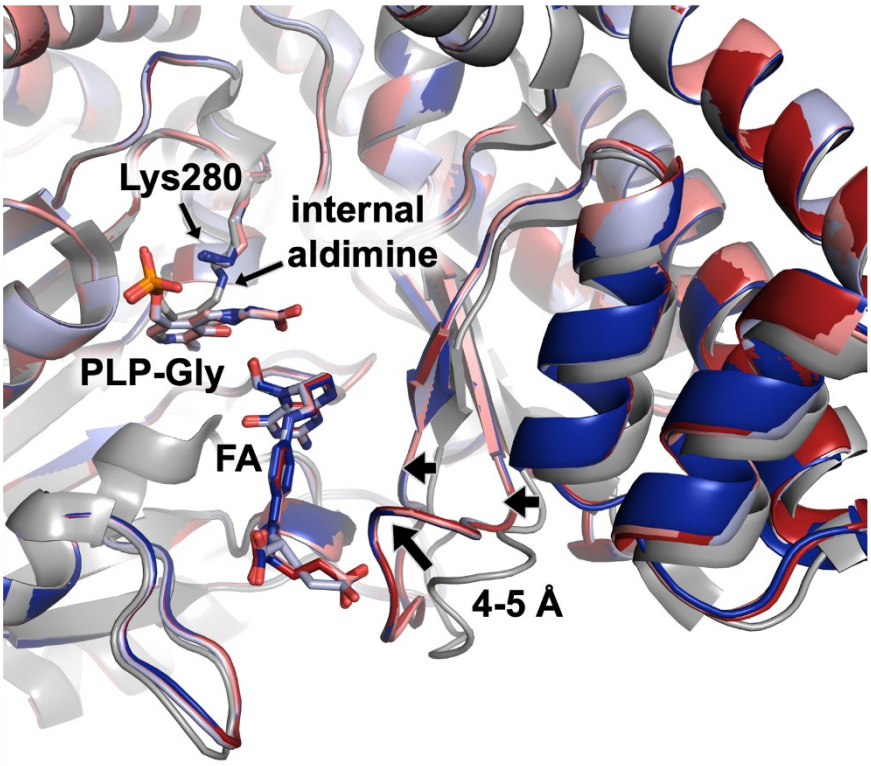


Figure S5. Overview of hSHMT2-Gly/FA gating loop closure in all protomers compared to the gating loop in protomer B in of our previous hSHMT2 holoenzyme structure (light grey, PDB ID: 8SSJ). In the hSHMT2-Gly/FA ternary complex, all the protomers (A = pink, C = red, B = light blue, D = dark blue) are virtually identical, superimposing with an RMSD on 0.14 ± 0.02 Å, and demonstrating a 4-5 Å shift upon FA binding to move the gating loop into a closed state.

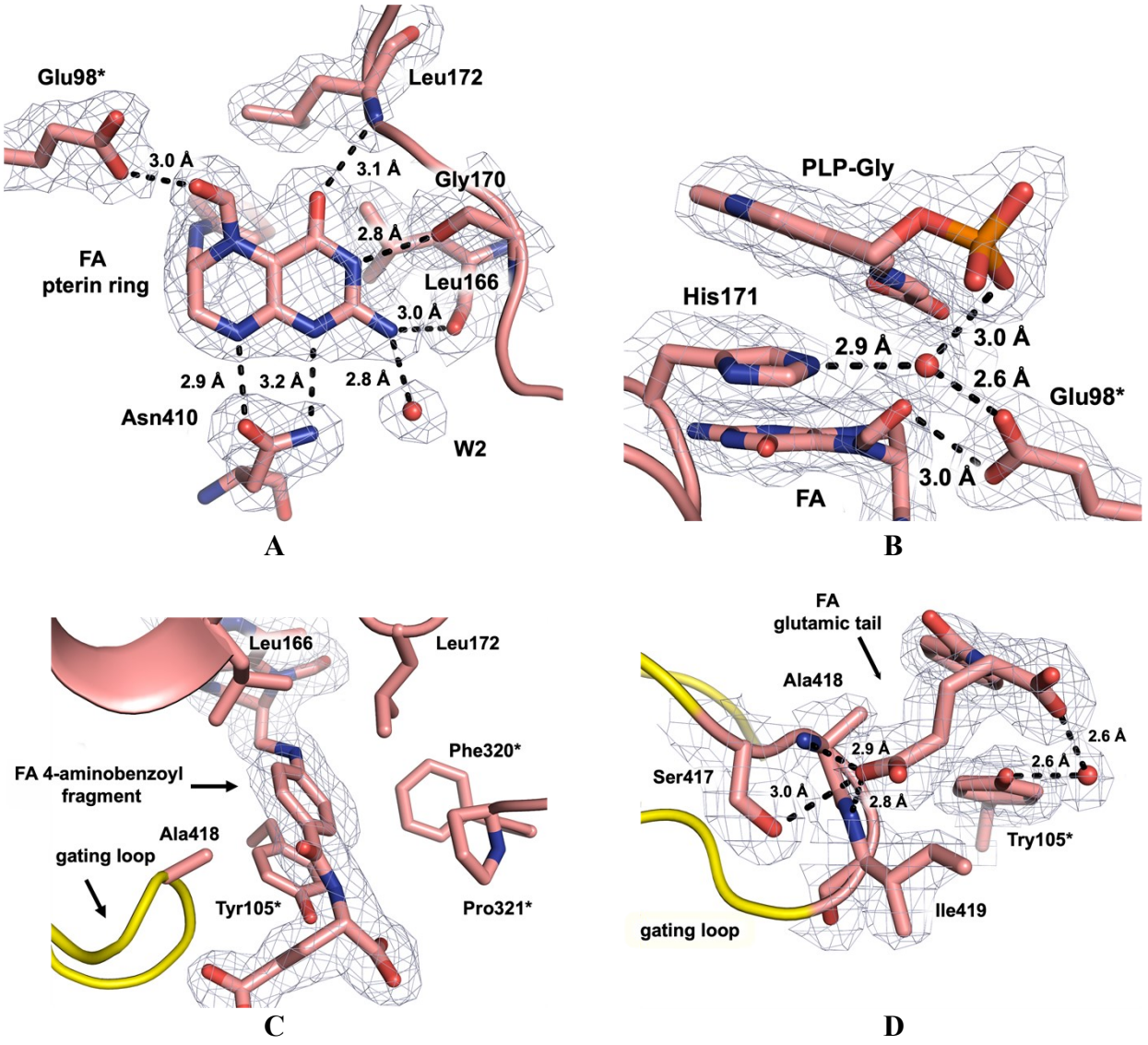


Figure S6. Binding of FA in hSHMT2-Gly/FA complex shown for protomer A. Interactions between FA and hSHMT2 active site residues are similar in the other protomers, B-D. a) The FA pterin moiety in hSHMT2-Gly/FA binds at the interface to the cationic substrate binding site, oriented by H bonds with Leu172, Gly170, Leu166, Asn410, Glu98*, and a water molecule. b) Glu98* makes an H bond with the FA N5 aldehyde oxygen atom and water molecule, W1. W1 is firmly held in position by additional H bonds with His171 and the PLP phosphate group. c) Leu166, Leu172, Phe320*, Pro321*, and Ala418 form a hydrophobic cage to position 4-aminobenzoyl group of FA in the peripheral binding site. d) The FA glutamate tail is flexible and extends out to the bulk solvent. The main chain carboxylate interacts with a surface water molecule and the gating loop stabilizes the side chain carboxylate through H bonds with the side chain of Ser417 and the backbones of Ala418 and Ile419. $2F_O$-F_C electron density maps are contoured at 1σ level in all panels.