Supporting Information

A flap motif in human serine hydroxymethyl transferase is important for structural stabilization, ligand binding, and control of product release

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List of Supplemental Materials

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1. Supplemental table

Table S1. Summary of the purification of the recombinant hcSHMT/Δflap.[a]

Purification step	Volume (mL)	Total protein (mg)	Total activity (unit)	Specific activity (unit/mg)	Purification fold
Crude extract	198	16,707	9,003	0.54	1
1% (w/v) PEI	170	7,786	6,028	0.77	1.43
30-50% (w/v) $(NH_4)_2SO_4$	80	6,410	4,652	0.73	1.35
DEAE-Sepharose	12.5	2,016	3,906	1.94	3.59
Sephacryl S-200	17	1,775	7,548[b]	4.25[b]	7.87

[a]This purification table is from 7.8 liters culture.
[b]This fraction was assayed after addition and removal of excess free PLP, respectively.

2. Supplemental figures with legends

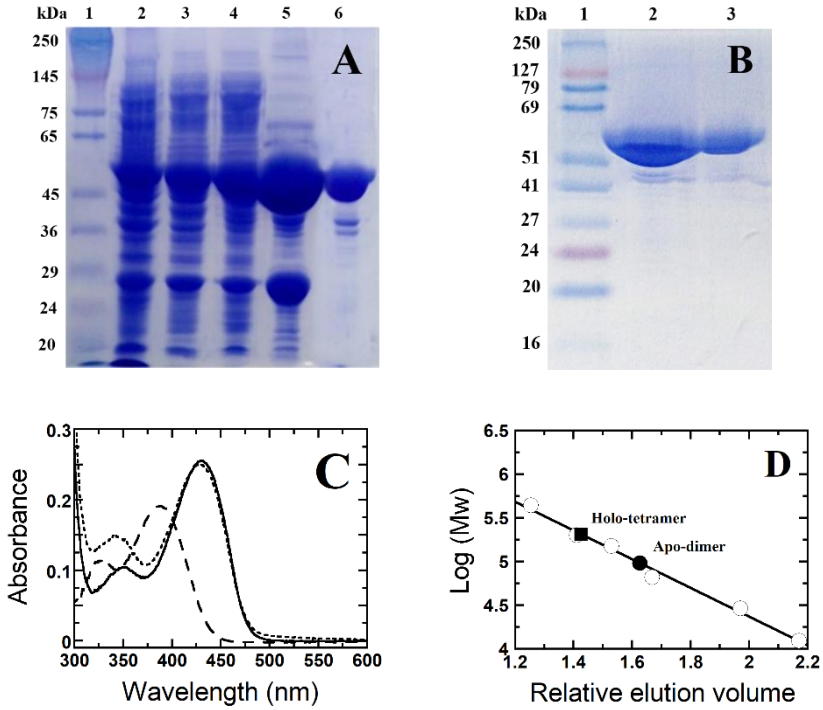


Figure S1. Characterization of the purified hcSHMT/Δflap. (A) 12% (w/v) SDS-PAGE analysis. Lane 1, AccuProtein markers (Enzmart Biotech); lane 2, crude extract; lane 3, extract after precipitation by 1% (w/v) polyethyleneimine; lane 4, after fractionation by 30-50% (w/v) ammonium sulfate; lane 5, after purification on a DEAE-Sepharose column; and lane 6, after purification on a Sephacryl S-200 column, respectively. The MW of the hcSHMT/Δflap monomer is approximately 52 kDa, consistent with the calculated MW. (B) 12% (w/v) SDS-PAGE analysis of the holo-tetramer (lane 2) and apo-dimer (lane 3) fractionated by SEC. (C) The overlaid absorption spectra of the hcSHMT/Δflap (solid line) and wild-type (dotted line) with maximum absorption at 430 nm, and the spectrum of free PLP (dashed line) with maximum absorption at 388 nm. (C) A calibration curve plot of the log MW of standard proteins (ferritin 440 kDa, β-amylase 200 kDa, alcohol dehydrogenase 150 kDa, bovine serum albumin 66 kDa and carbonic anhydrase 29 kDa, cytochrome c 12.4 kDa) *versus* relative elution volume. The native MW of holo-tetramer and apo-dimer are 206 and 96 kDa, respectively.

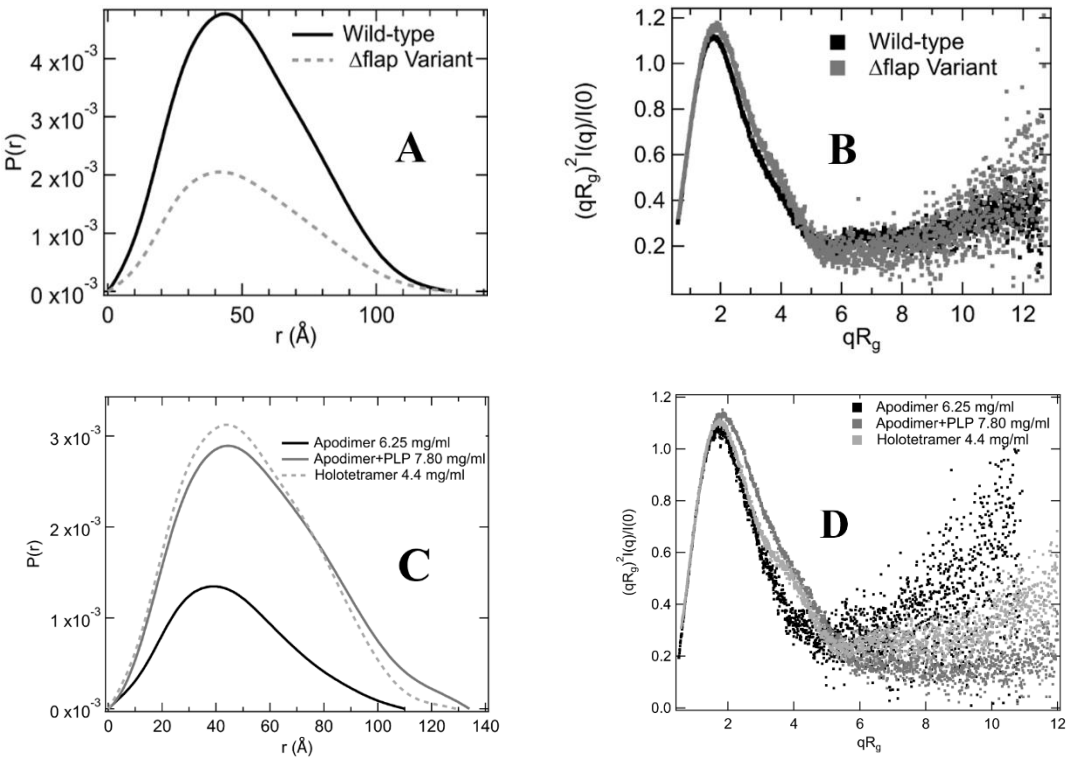


Figure S2. Pair distribution function (P(r)) and Kratky analyses. (A) Pair distribution function (P(r)) and (B) Kratky analysis of the hcSHMT/Δflap compared with those of the wild-type. (C) P(r) and (D) Kratky analysis of the apo-dimer, PLP-reconstituted apo-dimer and holo-tetramer of hcSHMT/Δflap.

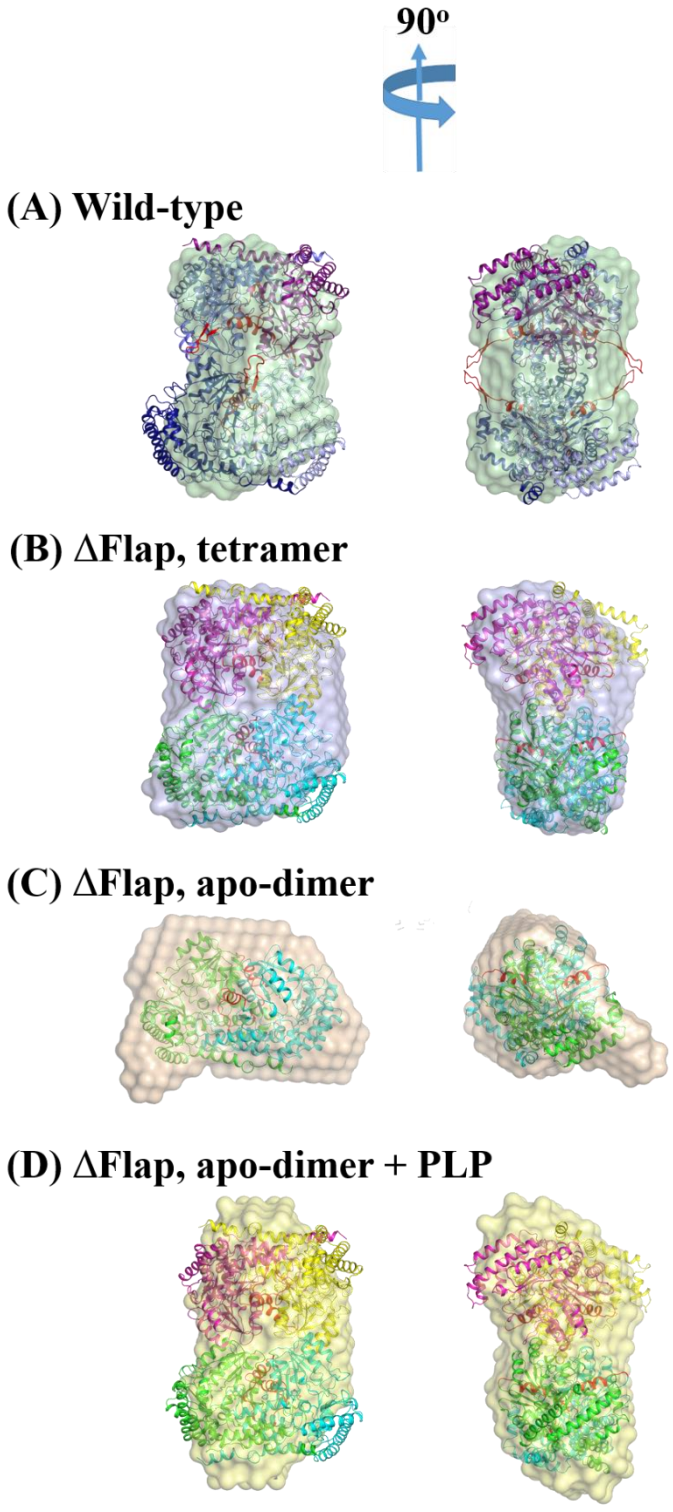


Figure S3. The *ab initio* models of the different oligomers of hcSHMT/Δflap compared to the wild-type derived from SAXS. (A) wild-type holo-tetramer, (B) Δflap holo-tetramers, (C) Δflap apo-dimer and (D) PLP-reconstituted tetramer after addition of PLP to the Δflap apo-dimer.

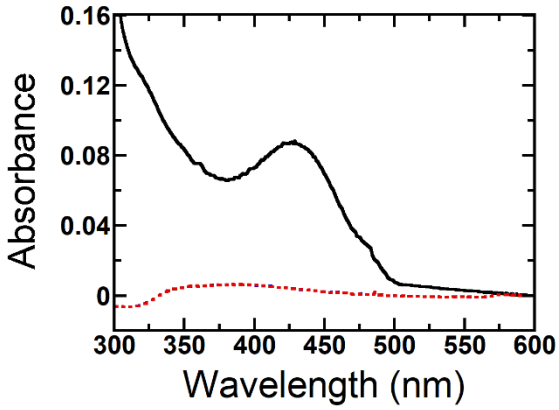


Figure S4. Absorption spectra of released PLP (dotted line) and hcSHMT/Δflap holo-tetramer (black solid line) to determine the equilibrium binding constant. The K_d of PLP was determined to be 0.228 ± 0.005 μM. The experiments were done in triplicate.

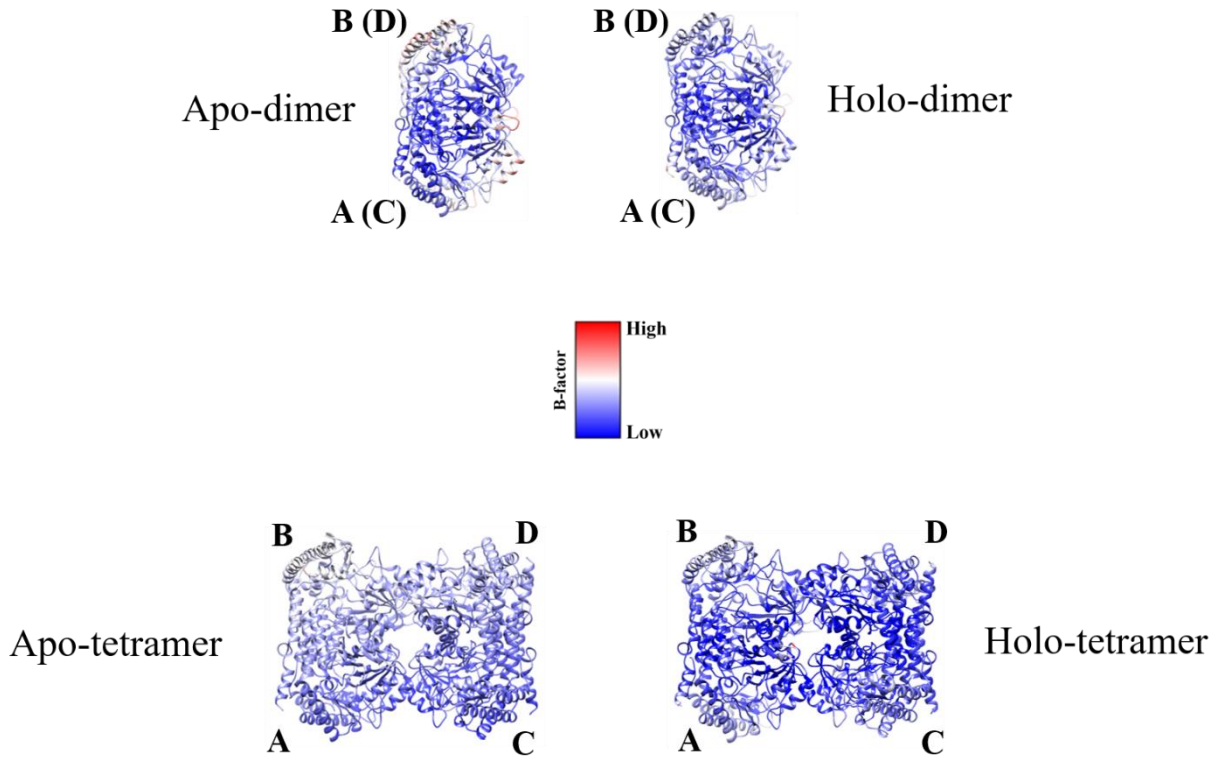


Figure S5. Molecular dynamics simulation of apo/holo forms of the dimer and tetramer of wild-type hcSHMT. The MD simulation results for the wild-type indicated that the thermal motion of the apo and holo forms of the dimer and tetramer of the wild-type were similar. This suggests the presence of tightly-packed domains in all oligomeric states of the wild-type, even though the obligate tetramer is the only form found in nature for the wild-type.

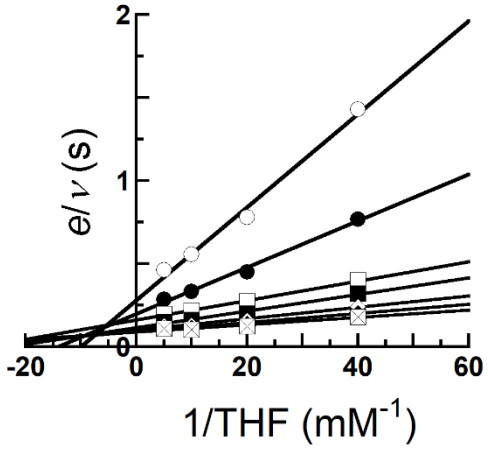


Figure S6. A Dalziel plot of the two-substrate kinetics of the hcSHMT/Δflap reaction at pH 7.5. A double-reciprocal plot of the initial velocities *versus* various concentrations of THF (0.025-0.2 mM) at various fixed L-serine concentrations (0.1-6.4 mM). A pattern of intersecting lines of a double-reciprocal plot indicates a ternary-complex kinetics of the hcSHMT/Δflap reaction.

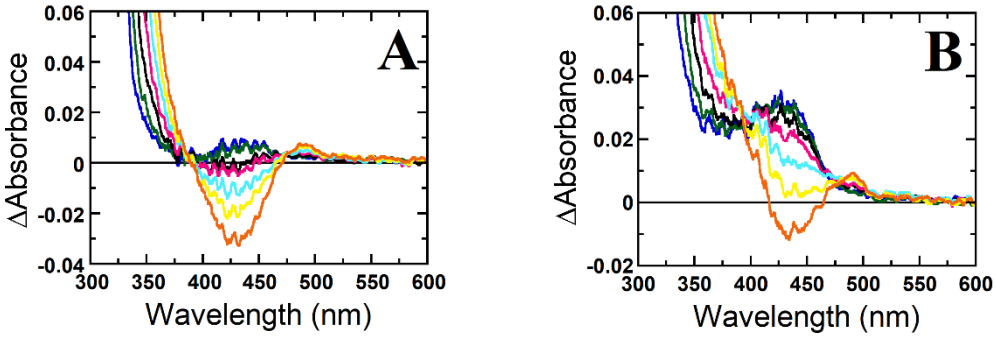
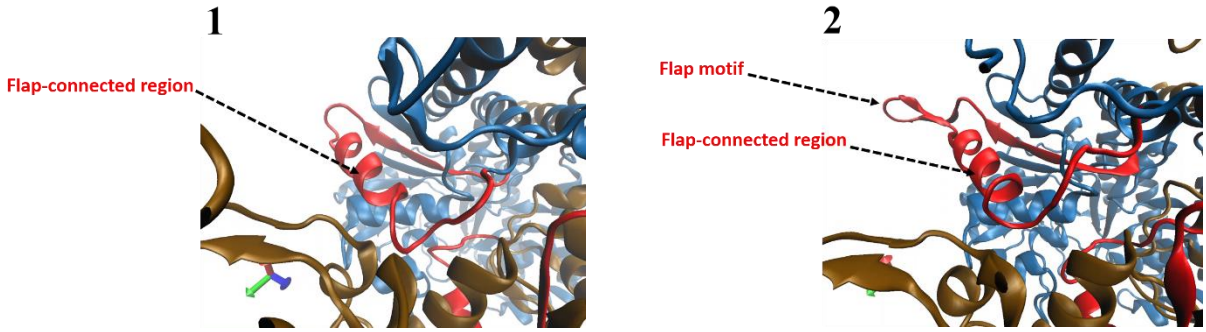
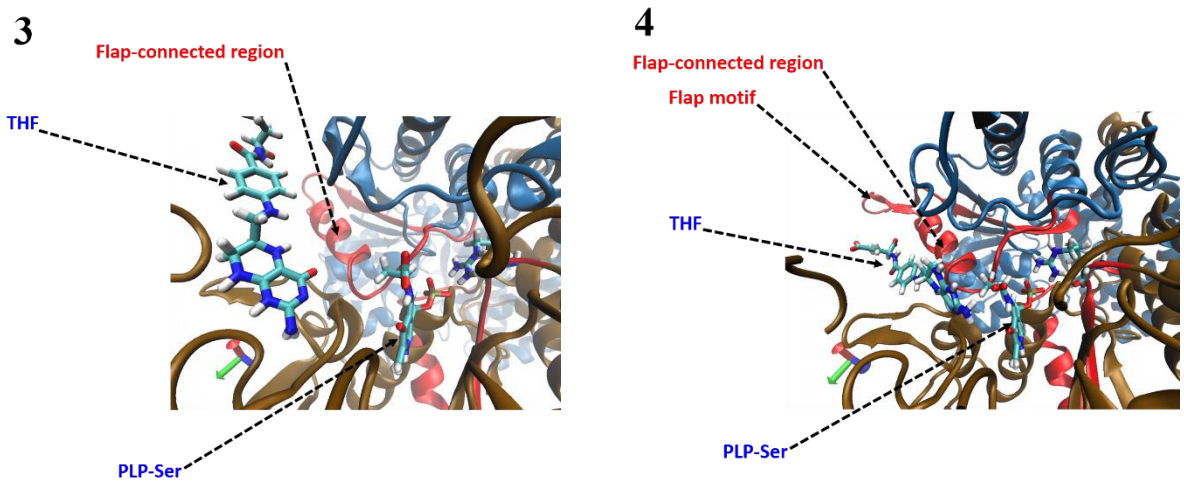


Figure S7. The difference spectra for the binding reactions of the (A) hcSHMT/Δflap and (B) wild-type with THF at pH 7.5.

3. Supplemental MD movies with legends



Movies 1 and 2. A graphical representation of a 100-ns MD simulation of the hcSHMT/Δflap (1) and wild-type (2) dimers in the presence of THF and PLP-Ser Schiff base. The movies 1 and 2 show protein dynamic and a conformational change of the flap-connected region with the ligands omitted. The flap-connected region is highly conformational dynamic in the hcSHMT/Δflap compared to the wild-type.



Movies 3 and 4. A graphical representation of a 100-ns MD simulation of the hcSHMT/Δflap (3) and wild-type (4) dimers in the presence of THF and PLP-Ser Schiff base. The movie 3 shows that THF can be easily released from the hcSHMT/Δflap. While the movie 4 shows that the flap motif promotes retention of THF in the binding pocket of the wild-type.