Crystal Structures of Cystathionine β-Synthase from *Saccharomyces cerevisiae*: One Enzymatic Step at A Time

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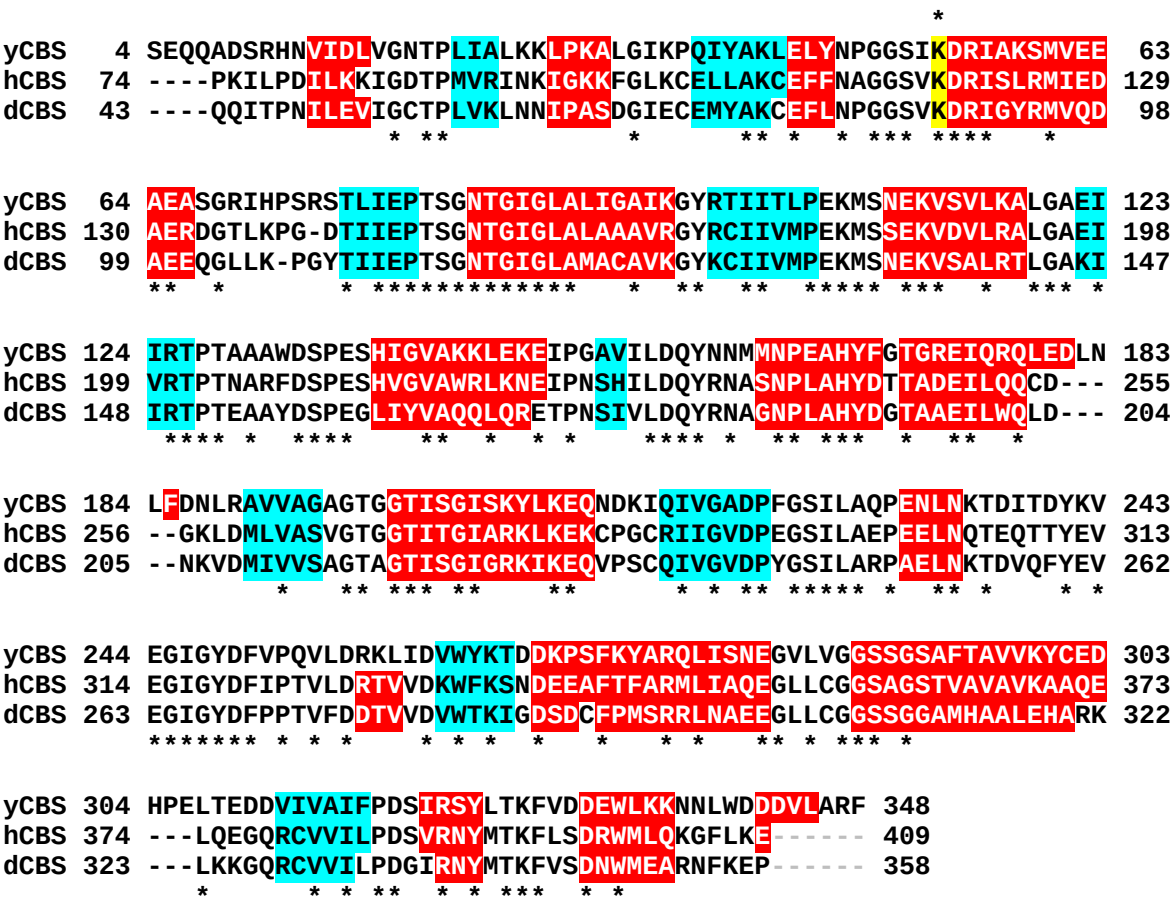

Figure S1. Structure-based sequence alignment of yCBS-cc (pdbid 6C2H) with hCBS (pdbid 4COO[1], chain A) and dCBS (pdbid 3PC2[2]) calculated using the Dali-server.[3] The catalytic domains for each enzyme are as follows: yCBS 14-329 (508 residues total), hCBS 80-386 (551 residues total), dCBS 49-355 (522 residues total). Residues in α-helices are colored red and residues in β-strands are colored cyan. The conserved lysine residue that makes a Schiff base with PLP is colored yellow and is marked by two asterisks. Throughout the alignment, identical residues are marked with an asterisk. Sequence identities for yCBS/hCBS and yCBS/dCBS are 52% over 326 residues aligned.

Table S1. Data collection and refinement statistics for yCBS-cc structures

	Catalytic Core PLP	+ L-serine PLP-L-ser	+ aminoacrylate PLP-aa	+ hydrazine PMP
Data collection				
Space group	$P6_522$	$P6_522$	$P6_522$	$P6_522$
Cell dimensions				
a, *b*, *c* (Å)	81.4, 81.4, 208.4	81.1, 81.1, 208.5	81.5, 81.5, 209.5	80.7, 80.7, 207.9
α, β, γ °	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120
Resolution (Å)[a]	50.0-1.49 (1.52-1.49)	50.0-2.17 (2.21-2.17)	50.0-1.37 (1.39-1.37)	50.0-2.30 (2.38-2.30)
Unique reflections	66342	22476	86357	17876
Completeness (%)	99.0 (100)	100 (100)	99.6 (99.5)	96.0 (99.2)
Redundancy	10.7 (9.4)	11.2 (9.5)	20.1 (12.6)	6.4 (6.4)
$I/\sigma I$	15.3 (2.1)	35.3 (9.0)	21.0 (1.5)	13.4 (7.6)
R_{merge}[b]	0.104 (0.880)	0.063 (0.277)	0.104 (≥1.0)	0.085 (0.195)
$CC_{1/2}$[c]	0.886	0.987	0.860	0.982
Refinement				
Resolution (Å)	26.4-1.49	34.7-2.17	26.5-1.34	31.0-2.30
No. reflections	61839 (3247)	21199 (1188)	75277 (4072)	17781 (1778)
R_{work}/R_{free}[d]	0.126/0.163	0.159/0.203	0.134/0.163	0.169/0.211
No. atoms				
Protein	2815	2730	2829	2691
Ligands	15, 4	22	21	16
Water	342	152	349	86
Mean *B* factors (Å^2)				
Protein	18.0	31.5	17.8	31.5
PLP	12.1	28.4	12.7	
PMP				40.6
Acetate	19.5			
Water	33.2	35.1	33.3	43.4
rms deviations[e]				
Bond lengths (Å)	0.013	0.009	0.012	0.012
Bond angles (°)	1.599	1.389	1.554	1.521
PDB code	6C2H	6C2Q	6C2Z	6C4P

[a] Values in parantheses are for the highest resolution bin

[b] $R_{merge} = \Sigma|I_{obs}-I_{avg}|/\Sigma I_{avg}$

[c] Values reported are for the highest resolution bin

[d] $R_{work} = \Sigma|F_{obs}-F_{calc}|/F_{obs}$, R_{free} calculated as R_{work} for a test set of reflections excluded from refinement

[e] rms deviations calculated by REFMAC[4]

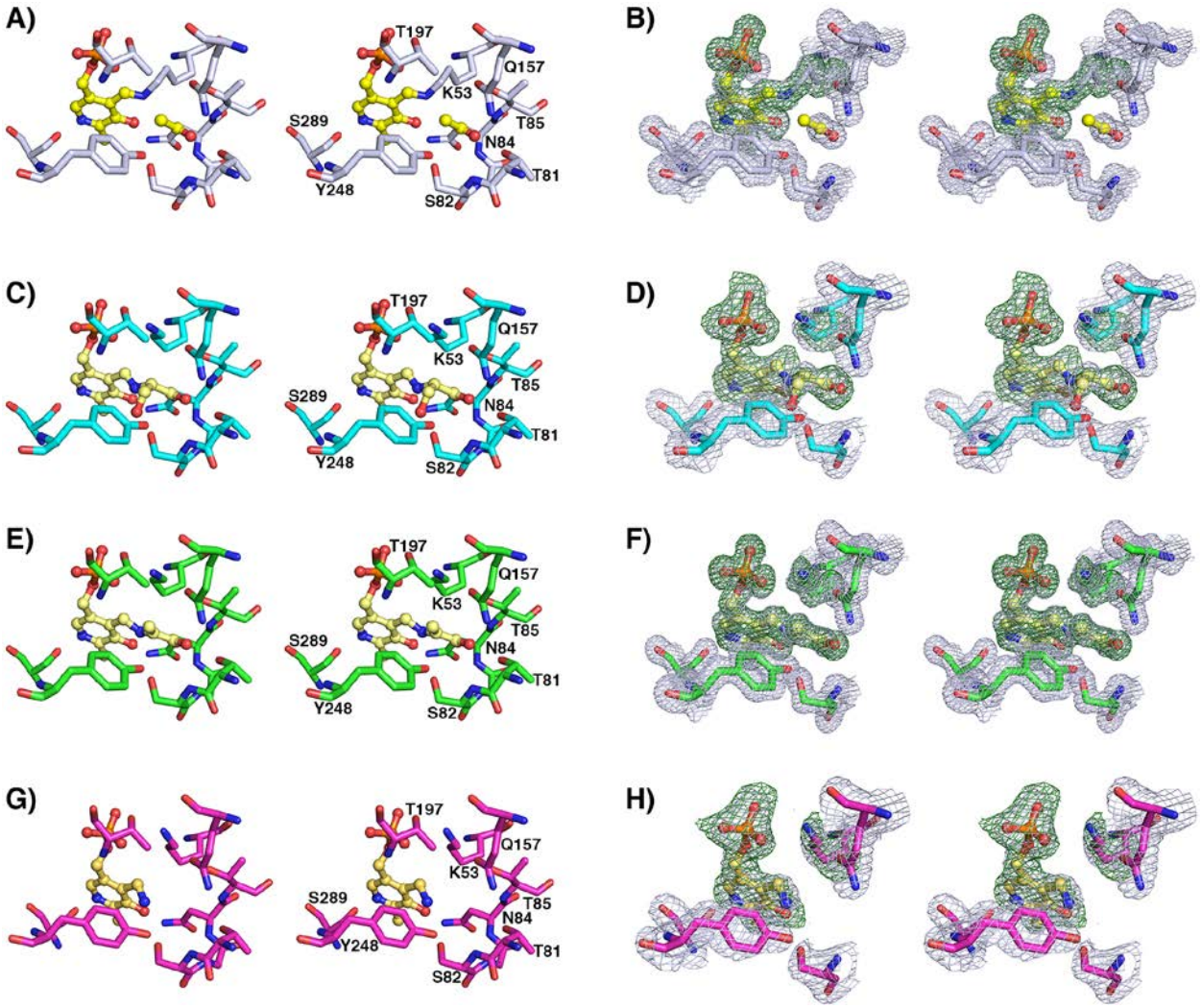

Figure S2. Electron density in the yCBS-cc active sites. (A, B) The PLP internal aldimine active site (PDB ID 6C2H). (C, D) The PLP-L-serine external aldimine intermediate active site (PDB ID 6C2Q). (E, F) The PLP-aminoacrylate external aldimine intermediate active site (PDB ID 6C2Q). (G,H) The PMP active site (PDB ID 6C4P). $2F_{obs} - F_{calc}$ electron density contoured at 1σ is shown in light blue. $F_{obs} - F_{calc}$ electron density contoured at 3σ (2.6σ for panel H) is shown in green and was calculated from a simulated annealing omit map from which the ligand and K53 were omitted during the process. Atoms are colored according to element as follows: grey, cyan green, or magenta, carbon; blue, nitrogen; red, oxygen; orange, phosphorus. The carbon atoms of PLP are colored yellow.

Table S2. Distances (Å) of interactions in the yCBS-cc active sites

	Catalytic Core	+ L-serine	+ aminoacrylate	+ hydrazine
yCBS Residue	**PLP**	**PLP-L-ser**	**PLP-aa**	**PMP**
S289-OG	N1 2.69	N1 2.82	N1 2.72	N1 2.78
N84-ND2	O3 2.77	O3 2.72	O3 2.79	O3 2.72
water*	O3 2.80	O3 2.71	O3 2.73	O3 2.79
S82-OG	N/A	N/A	N/A	NH_2 3.17
G196-NH	O1P 2.81	O1P 2.78	O1P 2.81	O1P 2.81
G198-NH	O1P 2.82	O1P 2.81	O1P 2.84	O1P 2.96
water	O1P 2.64	O1P 2.65	O1P 2.69	O1P 2.64
T197-OG1	O2P 2.70	O2P 2.73	O2P 2.66	O2P 2.59
T197-NH	O2P 2.86	O2P 2.90	O2P 2.87	O2P 2.85
K53-NZ	N/A	O2P 2.76	O2P 2.83	O2P 3.14
water†	O2P 2.98	N/A	N/A	N/A
T200-OG1	O3P 2.62	O3P 2.61	O3P 2.63	O3P 2.64
T200-NH	O3P 2.88	O3P 2.90	O3P 2.97	O3P 3.01
W’	O3P 2.76	O3P 2.69	O3P 2.73	O3P 2.81
water	O4P 2.80	O4P 2.75	O4P 2.83	O4P 2.95
	acetate	**L-ser carboxylate**	**aa carboxylate**	**W^{Aa}**
T81-OG1	OXT 2.51	OXT 2.62	OXT 2.57	2.87
S82-NH	OXT 2.87	OXT 2.95	OXT 2.95	3.45
S82-OG	OXT 3.42	OXT 3.46	OXT 3.44	2.97
Q157-NE2	OXT 2.83	OXT 2.85	OXT 2.83	4.57
T85-NH	O 2.88	O 2.99	O 2.85	3.02
N84-NH	O 3.15	O 3.20	O 2.97	3.20
water*	O 3.14	O 3.32	O 3.56	3.33
	W”[b]	**L-ser OG**	**W”**	**N/A**
S82-OG	2.77	2.64	2.83	
G245-O	3.37	3.43	3.05	
Y248-OH	2.62	2.97	2.65	
water†	2.60	N/A	N/A	
water	2.72	N/A	2.62	
	K53-NZ	**K53-NZ**	**K53-NZ**	**K53-NZ**
W’	N/A	2.61	2.91	2.47
O2P	N/A	2.76	2.83	3.14
Q157-OE1	N/A	3.46	3.08	3.69
	W’[c]	**W’**	**W’**	**W’**
K53-NZ	N/A	2.61	2.91	2.47
O3P	2.76	2.69	2.73	2.81
H167-NE2	2.87	2.87	2.90	2.70
N163-OD1	2.85	3.07	2.89	2.90
H167-NE1 to T171-OG1	2.79	2.87	2.81	2.84

[a] W^{A} Water occupying approximately the same location as an acetate or carboxylate oxygen atom
[b] W” Water indicating the position of the attacking sulfur
[c] W’ Water that coordinates K53 in the absence of the Schiff base linkage to PLP

Table S3. Kinetic parameters for yCBS[5]

	yCBS WT	yCBS C-terminal truncated
k_{cat} (s^{-1})	17.3 ± 0.8	16.0 ± 0.96
K_M(Ser)(mM)	11.23 ± 0.92	5.34 ± 0.64
K_M(Hcys)(mM)	0.615 ± 0.051	0.445 ± 0.059
k_{cat}/K_M (Ser)($s^{-1}M^{-1}$)	$1.45x10^3$	$2.97x10^3$

From Jhee, *et al.* (2000)[5]

Table S4. Unfolding transitions for yCBS

Protein	T_M (°C)
yCBS FL WT	57.5[a]; 59.0[b]
yCBS FL WT[c]	59.9
yCBS cc	59.2[a]; 55.8[b]
yCBS cc + SAM	54[a]
yCBS FL + SAM	51[b]
yCBS FL + SAM[c]	60.1
yCBS FL + D-cycloserine (1 mM)	40[b]
yCBS cc + D-cycloserine (1 mM)	40[b]
yCBS FL + hydrazine (1 mM)	46[b]
yCBS cc + hydrazine (1 mM)	45[a]; 48[b]
hCBS FL WT[c]	53 (regulatory domain) 71.4 (catalytic domain)
hCBS FL WT[d]	71.8 (peak at ~52.5 interpreted as unfolding of regulatory domain)
hCBS Δ414-551[c]	56 (marginal transition due to heme domain) 71 (catalytic domain)
dCBS FL WT[c]	70.8 (~55, possibly unfolding of heme-binding domain)

[a] CD measurement of yCBS (20 uM), this work
[b] ThermoFluor measurement of yCBS (20 uM), this work
[c] DSC measurement[6]
[d] DSC measurement[7]

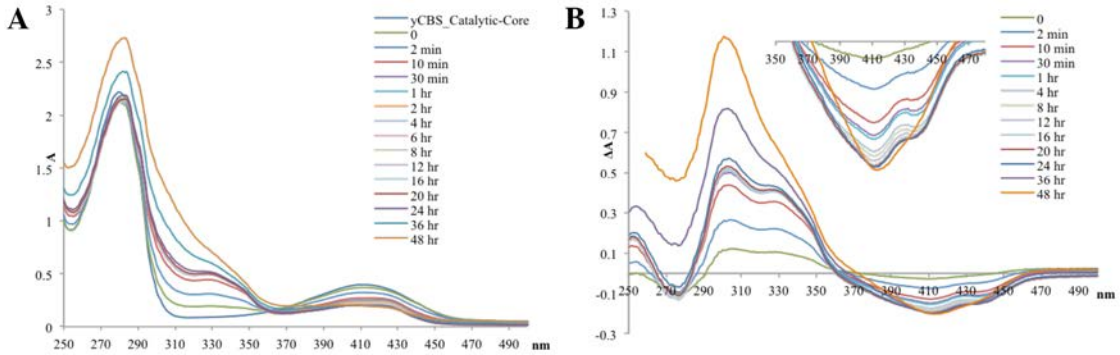

Figure S3. UV-vis absorption spectra of yCBS-cc with the hydrazine inactivator. A) Reaction of yCBS-cc with the hydrazine inactivator was followed by incubating the enzyme at 50 μM with 500 μM hydrazine in 50 mM Tris pH 8.0 at room temperature. B) Spectra of A normalized with the enzyme only.

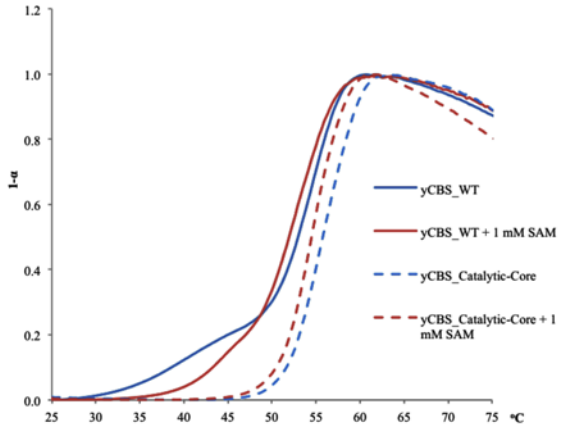

Figure S4. ThermoFluor assays of full-length yCBS (yCBS_WT) and yCBS-cc in the absence and presence of SAM. The protein concentration is 5 μM and the SAM concentration is 1 mM.

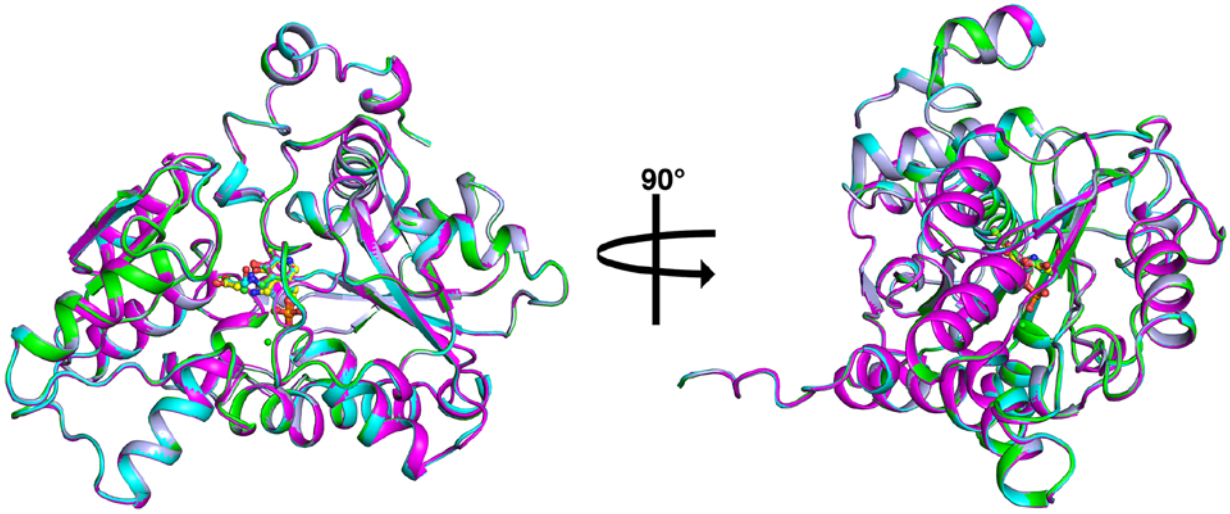

Figure S5. Stuctural alignment of all yCBS-cc structures presented in this work. The PLP internal aldimine structure is colored grey (PDB ID 6C2H), the PLP-L-serine external aldimine intermediate is colored cyan (PDB ID 6C2Q), the PLP-aminoacrylate external aldimine intermediate is colored green (PDB ID 6C2Z), and the PMP structure is colored magenta (PDB ID 6C4P). No gross structural changes are observed. The rmsd between pairs of structures as calculated by COOT[8] are as follows: internal aldimine-L-serine 0.111 Å; internal aldimine-aminoacrylate 0.090 Å; internal aldimine-PMP 0.179 Å; L-serine-aminoacrylate 0.123 Å; L-serine-PMP 0.144 Å; aminoacrylate-PMP 0.187 Å.

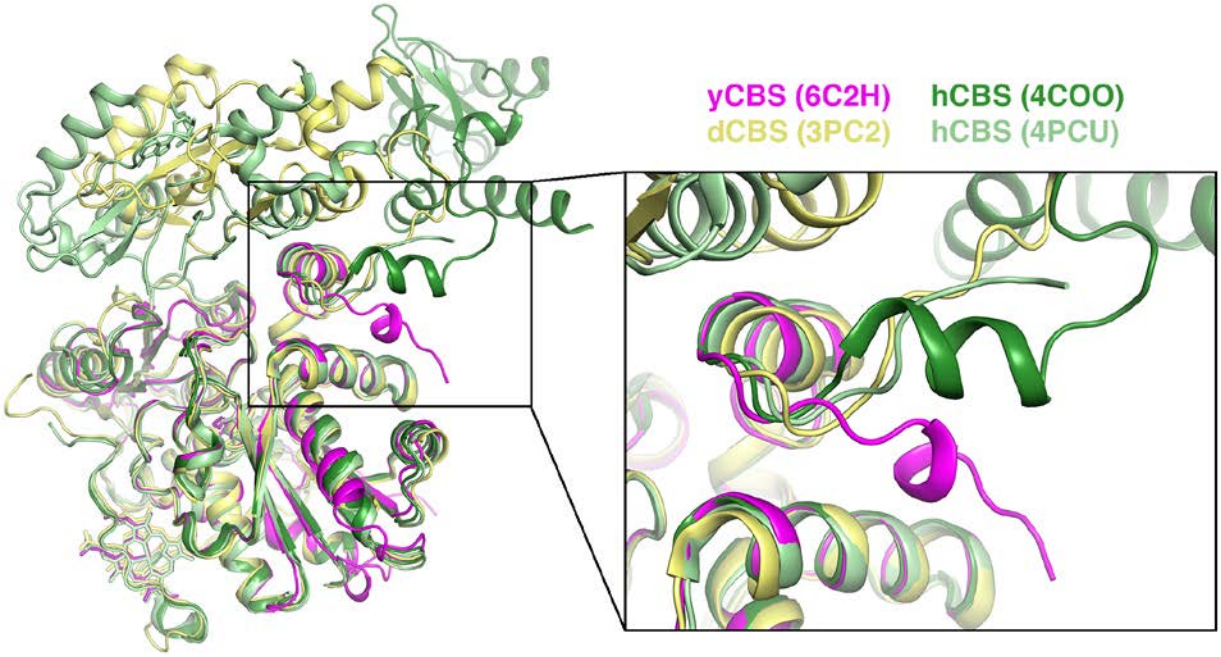

Figure S6. Structural alignment of yCBS-cc (magenta, PDB ID 6C2H), dCBS (light yellow, PDB ID 3PC2[2]), hCBS (dark green, PDB ID 4COO[1]), and activated hCBS (light green, PDB ID 4PCU[9]) highlighting the linker region at the carboxy terminus of yCBS shown in the boxed region. The catalytic core domain is located at the bottom of the figure and the regulatory Bateman module is at the top. The linker of the truncated yCBS-cc is orientated in a different direction compared to the linkers of the other three full-length CBS structures and suggests that the C-terminal regulatory domain might adopt a different orientation relative to the catalytic domain when compared to the regulatory domains observed in the structures of dCBS and hCBS.

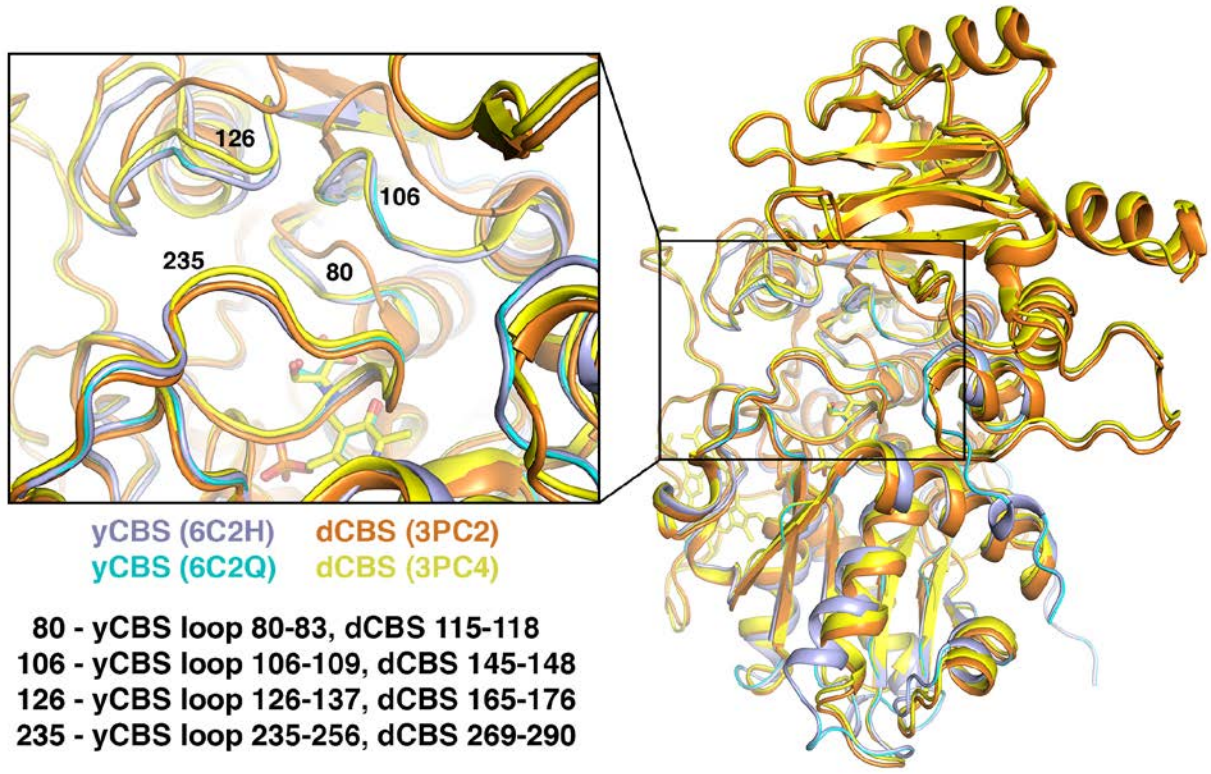

Figure S7. Structural alignment of the internal aldimine forms of yCBS-cc (grey, PDB ID 6C2H) and dCBS (orange, PDB ID 3PC2[2]) and PLP-L-serine external aldimine forms of yCBS-cc (cyan, PDB ID 6C2Q) and dCBS (yellow, PDB ID 3PC4[2]) highlighting loops near the active site. In dCBS, binding of substrate induces the loop containing S116 (yCBS S82) to shift toward the PLP cofactor. This change, along with movement of other loops in the region, effectively close access to the PLP site. In contrast, all of these loops in the yCBS-cc structures presented here adopt a "closed" conformation. Although not shown here, the aminoacrylate external aldimine forms of both yCBS-cc (PDB ID 6C2Z) and dCBS (PDB ID 3PC3[2]) also adopt a "closed" conformation with respect to these loops as do the internal aldimine forms of hCBS (PDB IDs 4L3V[10] and 4COO[1]). However, structures of both truncated forms of hCBS (PDB IDs 1JBQ[11] and 1M54[12]) and of the S-adenosylmethionine activated full-length hCBS (PDB ID 4PCU[10]) adopt an "open" loop conformation that is similar to that seen in the internal aldimine dCBS structure shown here.

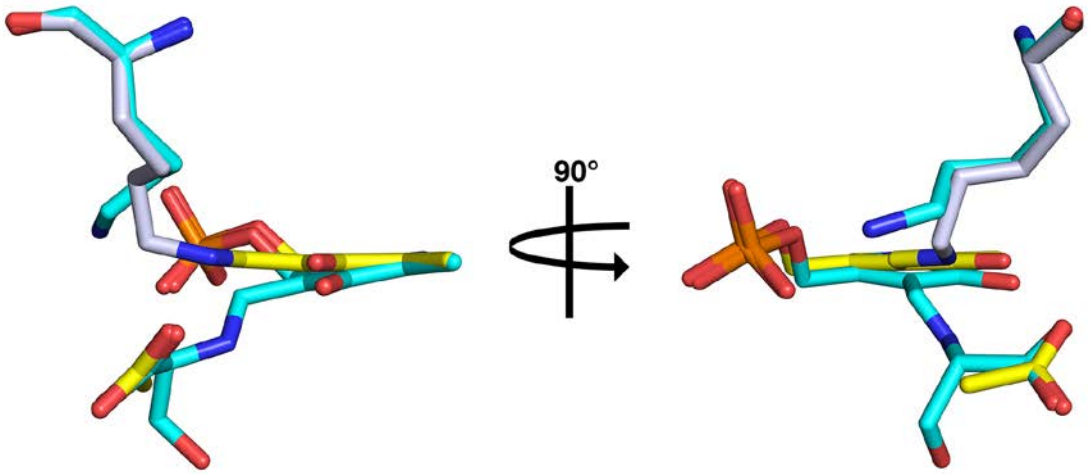

Figure S8. The PLP ring in the yCBS-cc PLP-L-serine external aldimine intermediate structure (cyan, PDB ID 6C2Q) is tilted approximately 11° compared to the yCBS-cc internal aldimine structure (yellow, PDB ID 6C2H). The pyridine ring adopts the same tilt in the aminoacrylate intermediate (PDB ID 6C2Z) and the PMP structures (PDB ID 6C4P). The carboxylates of both intermediates superimpose on the position of acetate observed in the internal aldimine structure.

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