Supporting Information

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SI Methods.

Expression and Purification of Wild Type and dCBS-ΔC144. Full-length *Drosophila* CBS (dCBS) was expressed from a bacterial expression construct (generously donated by Hadise Kabil and Scott Pletcher at the University of Michigan) that yields dCBS fused with glutathione *S*-transferase (GST). A truncated form of dCBS missing the C-terminal 144 amino acids, designated as dCBS-ΔC144, was generated by introducing a stop codon after C1134 in the dCBS cDNA using the following sense (5′-AC-CAGCGAATTCATGCCACAACCGAAGCC) and antisense primers (5′-CTCCGCGAATTCTCAGCTCCACCACCACCAG-TGACCG). The PCR product was digested with EcoR1 and cloned into the EcoR1 site of the expression vector pGEX-4T1. The sequence was verified by nucleotide sequence analysis (Sequencing Core Facility, University of Michigan). Proteins were expressed in *Escherichia coli* BL21 cells. Six liters of LB media, inoculated from an overnight culture, were supplemented with 450 μM δ-aminolevulinic acid and 150 μM pyridoxine and induced with IPTG (24 mg/L), at an OD_{600} of 0.5 and growth was continued at 28 °C for 14 h. Cell pellets were lysed in 200 mL of 50 mM Tris, pH 8, containing 1 mM *b*-mercaptoethanol, 10 mM EDTA, and complete protease inhibitor mix (Roche). Cells were lysed by sonication and centrifuged at 10,000 × *g* for 15 min. The supernatant was loaded onto a GST affinity column and washed with PBS, and the fusion protein was eluted with 10 mM glutathione. dCBS protein was liberated from GST by thrombin digestion and the two proteins were separated on a Q-sepharose anion exchange column as described previously (1).

Stopped-Flow Experiments. Stopped-flow experiments were performed on an Applied Photophysics stopped-flow spectrophotometer with a photodiode array detector. The temperature of the mixing chamber was maintained at 22 °C. A solution containing dCBS (30 μM, after mixing) in 100 mM Hepes buffer, pH 7.4, was mixed with serine (15 mM, after mixing) in the same buffer. Time-dependent formation of intermediates was followed by difference spectroscopy [(enzyme + serine) − (enzyme)].

Crystallization and Cryoprotection. Two reservoir solutions were used to afford crystals of substrate-free dCBS that were of similar diffraction quality: (*i*) 23% wt/vol PEG 3350, 0.2 M Li_2SO_4, 0.1 M [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane (Bistris), pH 6.5 and (*ii*) 20% wt/vol PEG3350, 0.2 M KCl. The resulting crystals were not single and special care was needed to harvest single crystals from larger aggregates. Crystals of substrate-free dCBS were cryoprotected for a few minutes prior to flash freezing in liquid N_2 by transfer to a solution of 17.5% wt/vol PEG3350, 0.17 M KCl (or Li_2SO_4), 0.05 M Bistris 7.0 and 10% vol/vol glycerol. substrate-free dCBS crystals were of space group $C222_1$ ($a = 92.9$, $b = 138.2$, $c = 75.2$, $\alpha = \beta = \gamma = 90.0$) with one monomer in the asymmetric unit.

Cocrystallization of dCBS with L-serine or with L-cysteine was not successful. However, we obtained crystals of the substrate complexes through soaking. Crystals of substrate-free dCBS obtained as described above were transferred to solutions that contained L-cysteine or L-serine. Specifically, substrate-free dCBS crystals were soaked for 17 h in solutions containing 17.5% wt/vol PEG3350, 0.05 M Bistris pH 7.0, 0.17 M KCl, 10% vol/vol glycerol, 10 mM L-cysteine or 10 mM L-serine. Crystals were then harvested directly from the drop and were flash frozen in liquid N_2. Soaking of substrate-free dCBS crystals with L-serine under slightly acidic conditions (17.5% wt/vol PEG3350, 0.05 M Bistris pH 6.5, 0.17 M KCl, 10% vol/vol glycerol) resulted in the aminoacrylate bound species.

1. Taoka S, Ohja S, Shan X, Kruger WD, Banerjee R (1998) Evidence for heme-mediated redox regulation of human cystathionine beta-synthase activity. *J Biol Chem* 273:25179–25184.

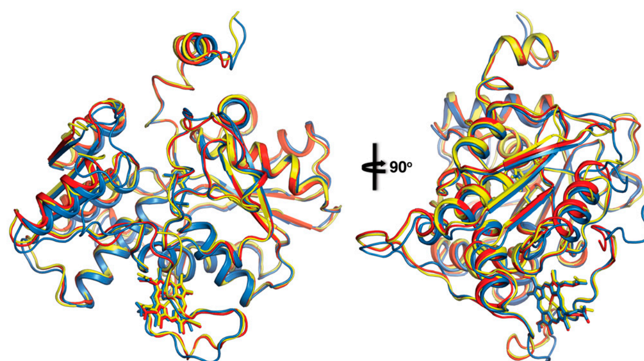

Fig. S1. Structural alignment of the pyridoxal phosphate (PLP) domains from two crystal structures of hCBS (red and yellow) and dCBS (blue). The PLP domains are highly similar. Three hundred forty Cα atoms of dCBS (P24-F370) align with hCBS [1JBQ (1)] with an rmsd of 1.0 Å, and with hCBS [1M54 (2)]with an rmsd of 1.15 Å. The rmsd for the alignment of the two hCBS structures is 0.61 Å. The loop between β_B3 and α_B5 was not modeled in the hCBS structures of the PLP domains; however, there is clear electron density in the dCBS structure for the equivalent region (residues T162-E170) that allowed us to build the loop between β_B3 and α_B5.

1 Meier M, Janosik M, Kery V, Kraus JP, Burkhard P (2001) Structure of human cystathionine beta-synthase: A unique pyridoxal 5′- phosphate-dependent heme protein. *EMBO J* 20:3910–3916.

2 Taoka S, et al. (2002) Human cystathionine beta-synthase is a heme sensor protein. Evidence that the redox sensor is heme and not the vicinal cysteines in the CXXC motif seen in the crystal structure of the truncated enzyme. *Biochemistry* 41:10454–10461.

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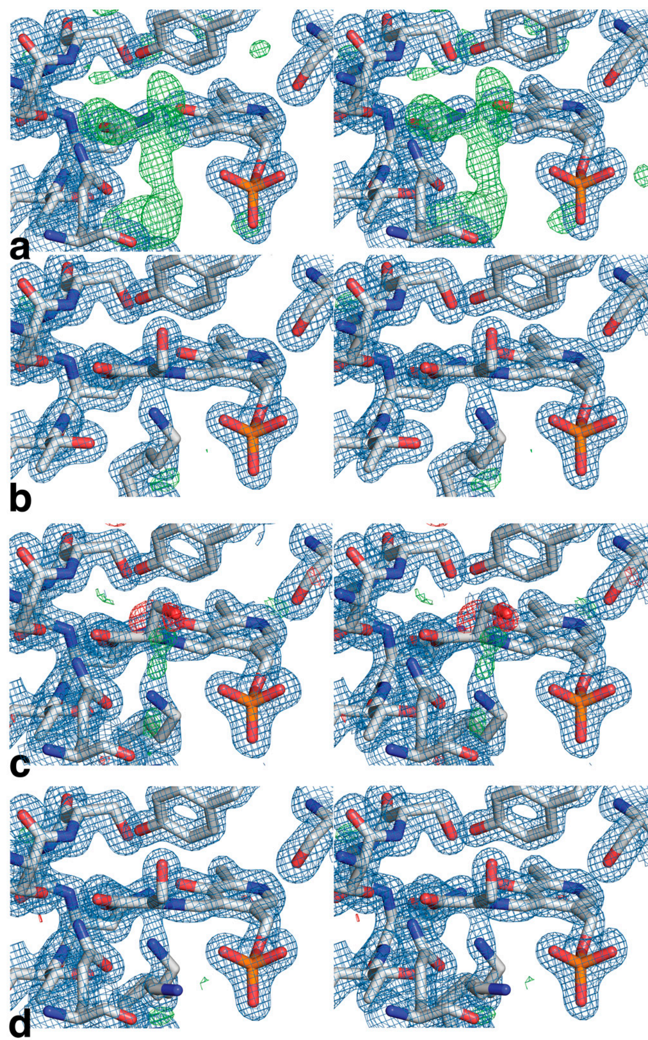
Fig. S2. Interactions between residues H372 and H374 in the linker of one subunit with CBSD1* residues from the other subunit form part of the tight dimer interface in the C-terminal region. The CBSD1* residues that contact the two histidine amino acids of the linker are shown in stick representation on the left and in a surface model on the right.

Fig. S3. (*A*) Structural changes elicited by substrate binding. (*B*) Close-up of the active site. The apo- and substrate-bound structures are shown in blue and yellow, respectively. A significant structural change involves loop L_B between strand β_B1 and helix α_B3, which relocates closer to the PLP cofactor.



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e

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g

Fig. S4. Cross-eyed stereoviews of electron density maps and stick models showing the active site geometry of dCBS with (*A*) no modeled serine intermediate or K88 side chain, (*B*) serine modeled as a carbanion, (*C*) serine with tetrahedral geometry around Cα, (*D*) serine modeled as a carbanion and K88 side chain modeled in two distinct conformations at different occupancies. In *D* the second conformation of the K88 side chain is similar to the one seen in the aminoacrylate bound complex. In *E*, serine is modeled as a carbanion and the K88 side chain has been omitted (modeled as K88G). In *F*, serine is modeled with tetrahedral geometry around Cα (tetrahedral intermediate 2), whereas the K88 side chain has been omitted (modeled as K88G). In *G*, serine is modeled as in *F* but with the K88 side chain present. The blue contours at 1.5σ represent electron density from a weighted $2F_{obs} - F_{calc}$ map. The green contours at 3σ represent positive electron density, whereas the red contours at -3σ represent negative electron from a $F_{obs} - F_{calc}$ map. Atom color coding is as follows: gray, carbon; blue, nitrogen; red, oxygen; orange, phosphorous.

Fig. S5. Sequence comparison of dCBS and hCBS. Sequence alignment of hCBS and dCBS. The two species share 51% sequence identity. Over 30 sequences were aligned with clustal W (1), but only the hCBS and dCBS sequences are displayed here.

1 Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680.

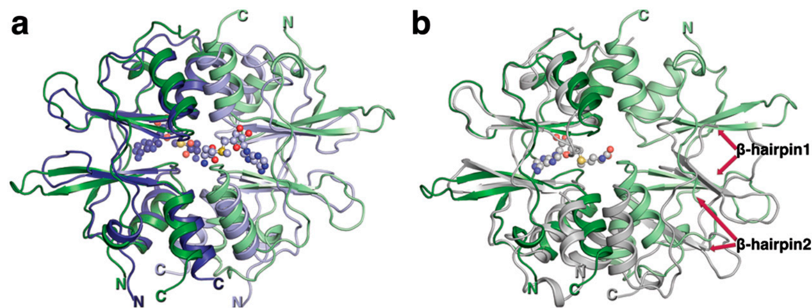

Fig. S6. Structural superimposition of the dCBS CBSD dimer (dark and light green) with two AdoMet-binding CBSD proteins: (*A*) 3KPC (1) (in dark and light blue), where AdoMet is bound in a closed conformation, and (*B*) 2YZQ (in white), where AdoMet is bound in an open conformation. These examples also exhibit two different modes of AdoMet binding: (*A*) AdoMet is bound in a conformation where the methionine part of the molecule is bent, and (*B*) AdoMet is bound in an extended conformation. The CBSD dimer from dCBS resembles the closed conformation of 3KPC, and the rmsd value on the basis of the Cα for these two proteins is 1.42 Å. However, whereas in dCBS and hCBS the equivalent AdoMet-binding regions are symmetrically across from each other (see Fig. 2*A*), in 3KPC, the AdoMet-binding regions are diagonally arranged. The differences in the arrangement of the AdoMet binding site are because of the fact that 3KPC is a symmetric dimer (CBSD1 interacts with CBSD1*, and CBSD2 interacts with CBSD2*). Modeling of two AdoMet molecules in dCBS or hCBS in a closed conformation is feasible only if AdoMet is in a bent conformation, as seen in 3KPC. If two AdoMet molecules are modeled in an extended conformation, as in 2YZQ, then the methionine moieties of AdoMet would clash with one another.

1 Lucas M, et al. (2010) Binding of S-methyl-5′-thioadenosine and S-adenosyl-L-methionine to protein MJ0100 triggers an open-to-closed conformational change in its CBS motif pair. *J Mol Biol* 396:800–820.

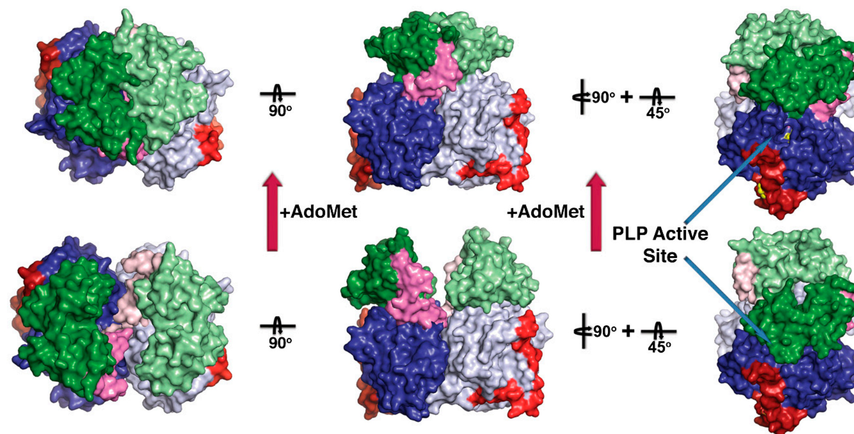

Fig. S7. Proposed model of CBS in the presence and absence of AdoMet, where dimerization is induced in the presence of AdoMet resulting in CBSD domain closure.