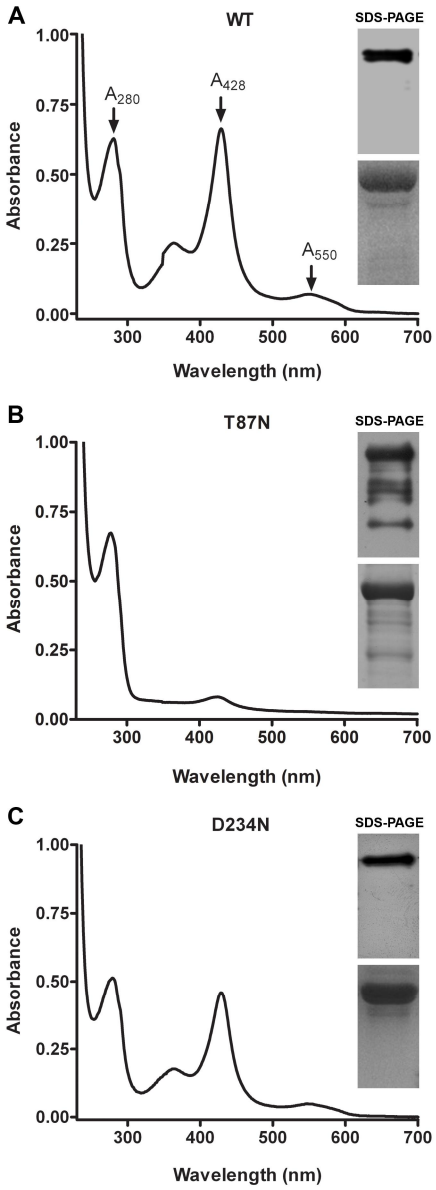


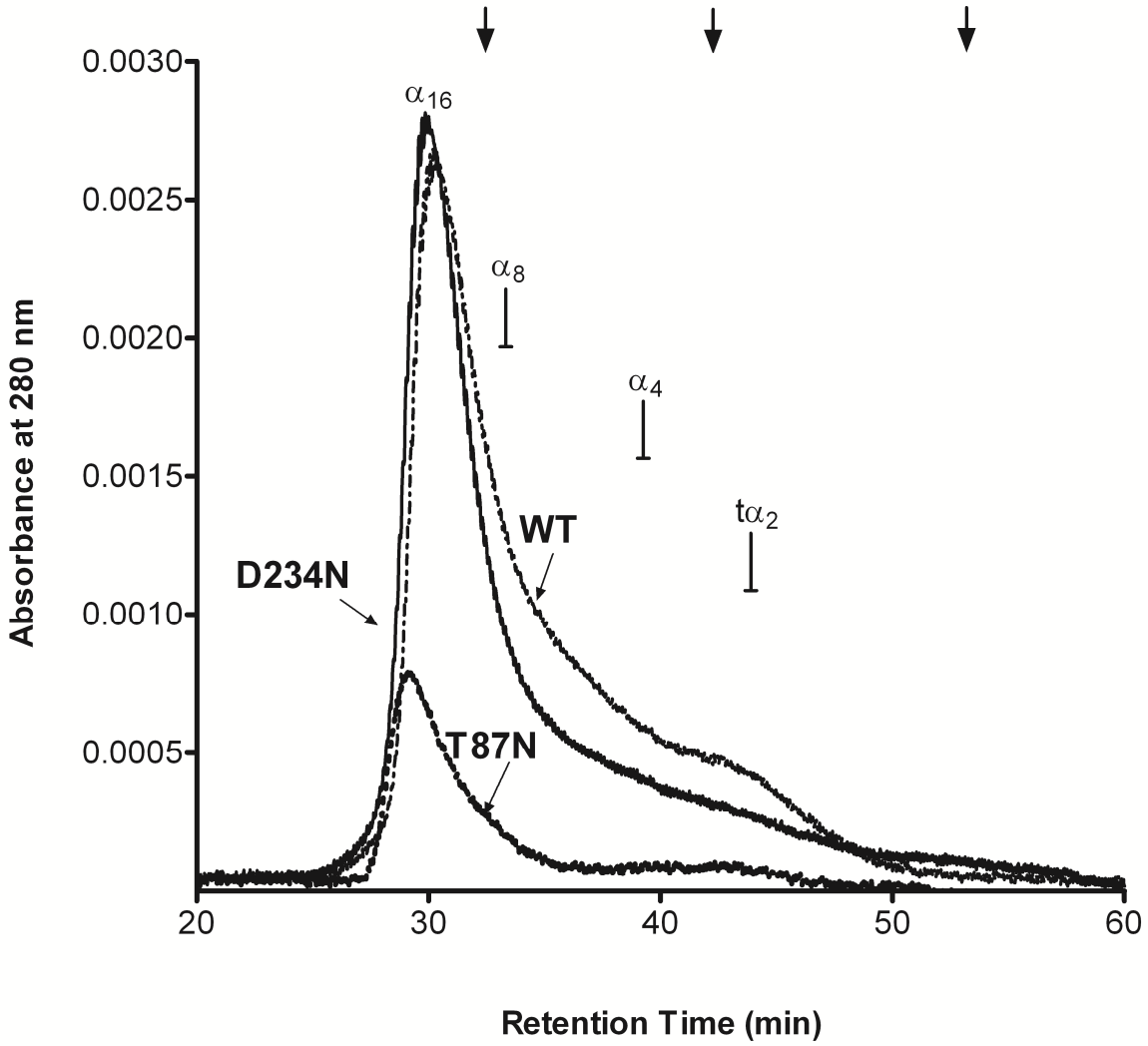
Supporting Figure 1: UV-visible spectra from purified CBS proteins. Absorption spectra from wild-type (A), T87N (B) and D234N protein. Insets show SDS-PAGE analyses of purified enzymes. Gels were visualized with Coomassie blue staining (bottom) or transferred to nitrocellulose membrane and probed with polyclonal anti-human CBS antibodies (upper). The D234N protein exhibited similar spectroscopic properties than native enzyme, showing the characteristic absorbance peaks from CBS proteins (indicated with arrows in A). Moreover, it was purified with a similar yield to the wild-type enzyme. The T87N mutant showed a decrease in 428 nm absorbance peak, indicating a lower saturation of the enzyme with heme. Although full-length enzyme represents around 70% of the sample, different low molecular weight bands were observed in SDS-PAGE gels, these bands correspond to degraded CBS polypeptides according to Western blot analysis of the purified enzyme.

Supporting Table 1: Kinetics parameters for D234N CBS

	D234N		Wild-type[c]	
	- SAM	**+ SAM**	**- SAM**	**+ SAM**
$\mathbf{K_{M[Hcy]}}$ **(mM)**[a]	4.0 ± 1.2	10.5 ± 4.5	4.8 ± 0.5	5 ± 0.9
$\mathbf{V_{max}}$ **(μmol/h/mg)**	130 ± 17	255 ± 45	178 ± 5	378 ± 17
$\boldsymbol{k_{cat}}$ **(s^{-1})**[b]	1.8	4	3	6.6
$\mathbf{K_{M[Ser]}}$ **(mM)**[1]	6.3 ± 0.9	6.3 ± 2.5	2 ± 0.3	2 ± 0.3

[a]K_M values were obtained by Michaelis-Menten analysis. The concentration of serine was 30 mM when homocysteine was varied (0-50 mM), and the concentration of homocysteine was 30 mM when serine was varied (0-30 mM). [b]k_{cat} was calculated based on molecular mass of the monomer. [c]Reported values for wild-type CBS are from Taoka and cowokers (1998) and Sen and Banerjee (2007).

The kinetic parameters for the D234N mutant are shown in Table 1. In the absence of SAM, the K_M for homocysteine was unaffected while the K_M for serine was ~3-fold higher than wild-type CBS. The addition of SAM to the reaction mixture increased both the K_M for homocysteine and the k_{cat} value by ~2-fold, while the K_M for serine was unchanged. Furthermore, both V_{max} and k_{cat} values were lower than reported for wild-type CBS.



Supporting Figure 2: Gel filtration profiles from recombinant CBS proteins showing the quaternary structures identified. Samples were loaded onto a Superdex-200 column with 50 mM TrisÅHCl buffer pH 8, 100 mM KCl at 4 ºC. Arrows on top of the figure represent elution positions for the following protein standards: thyroglobulin (670 kDa), bovine ɔ-globulin (158 kDa), and chicken ovalbumin (44 kDa) (Bio-Rad, Hercules, CA). The quaternary structures identified are indicated as oligomeric complex (α_{16}), octamer (α_8), tetramer (α_4), and truncated dimer ($t\alpha_2$).