Supporting Information For

## The Effect of the Disease-Causing R266K Mutation on the Heme and PLP Environments of Human Cystathionine β-Synthase

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**Figure S1.** Resonance Raman spectra of Fe(III) A) WT hCBS and B) R266K hCBS. Proteins (141  $\mu$ M and 235  $\mu$ M, A and B, respectively) were in 100 mM CHES buffer and 100 mM NaCl, pH 8.6. Spectra were acquired using solution samples by excitation with a 413.1 nm line provided by a Kr<sup>+</sup> laser with 10.5 mW of power at the sample. All measurements were carried out with the sample immersed in a bath of ice water to reduce local heating. Peak positions were calibrated against a K<sub>2</sub>SO<sub>4</sub> standard.



**Figure S2.** Arrhenius plots of the fit rate constants (min<sup>-1</sup>) for the loss of the Cys(thiolate)ligated heme Soret of Fe(II) R266K hCBS. Experimental values of  $k_1$  (solid circles, •) and  $k_2$ (solid squares, •) were determined for 10, 20 and 30°C (283, 293, and 303 K, respectively). Extrapolated values for  $k_1$  (open circle,  $\bigcirc$ ) and  $k_2$  (open square, ) were determined at 37°C (310 K). Error bars represent ± one standard deviation of the fit value determined from three replicate measurements.